TECHNOLOGY DEPARTMENT

Vol. XXVI July, 1949 No. 4

CEREAL CHEMISTRY

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Published bimonthly by the American Association of Cereal Chemists
at Prince and Lemon Sts., Lancaster, Pa.

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EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

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Entered as second-class matter March 3, 1932, at the post office at Lancaster, Pa., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in Section 1193, Act of October 3, 1917, authorized February 16, 1924.

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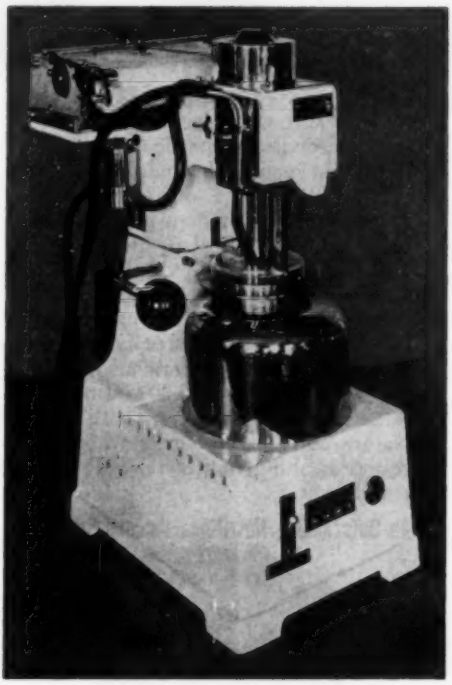

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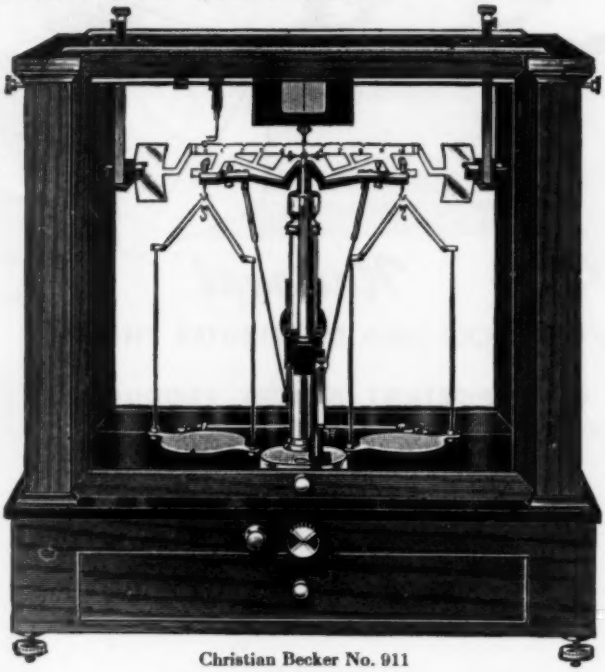

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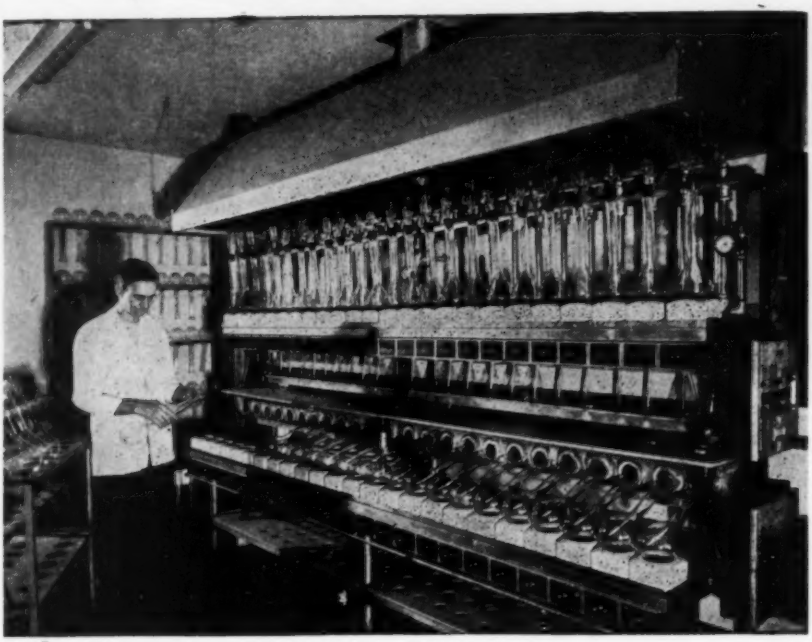

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CEREAL CHEMISTRY

VOL. XXVI JULY, 1949 No. 4

FACTORS CAUSING THE CHECKING OF MACARONI [1]

PAUL L. EARLE [2] and NORMAN H. CEAGLSKE [3]

ABSTRACT

The modulus of rupture, modulus of elasticity, coefficient of thermal expansion, and coefficient of moisture shrinkage of macaroni products were determined. The moisture gradients present during the drying were also measured and these data have been applied to compute the stresses present in macaroni. For commercial products the coefficient of thermal expansion was found to be 54×10^{-6} inches per inch—°C.; the coefficient of moisture shrinkage varied linearly with moisture content from 0.003 inch per inch—per cent moisture at 6% moisture to 0.0014 at 26% moisture; the average modulus of rupture of commercial spaghetti was 5,430 lb. per square inch.

The thermal stresses are small compared to the moisture stresses and to the strength of the macaroni. Plastic flow (creep) occurs at high moisture levels, relieving the stress which accompanies the formation of a moisture gradient. Reduction of the moisture gradient towards the end of drying sets up tensile stresses in the interior and compressive stresses at the outer surface which are sufficient to cause checking. Changes in relative humidity of the surroundings may produce moisture gradients due to the loss or gain of water which if sufficiently large will produce checking. The checks originate at the points of highest tensile stress.

A good macaroni has been defined as a product having the characteristics of hardness, brittleness, translucency, elasticity, and a rich amber color. The fracture should be glassy and long pieces should be sufficiently pliable to allow considerable bending before breaking.

The drying of macaroni is the most important operation in macaroni manufacture since the quality of the finished product largely depends upon the skill and judgment with which it is carried out (12). Some fundamental studies (9) on sheet macaroni dried at constant drying conditions show that macaroni exhibits a typical drying rate curve. The constant rate period ends at the critical moisture content of approximately 15% free water and is followed by the falling rate period.

Commercial drying (14) is divided into three stages, the preliminary

[1] Manuscript received February 18, 1949.

[2] Present address: Johns-Manville Corporation, Manville, New Jersey.
[3] Professor of Chemical Engineering, University of Minnesota.

drying, remoistening or sweating, and the final drying. Preliminary drying should reduce the water content from 45% (dry basis) to approximately 29–31% in 30 to 60 minutes depending upon the shape. This drying stabilizes the shape of the macaroni, prevents mold formation, and shortens the total time of drying. Remoistening or sweating consists of stopping the drying by an increase in the humidity. This allows the moisture to become uniformily distributed in the macaroni. In the final drying the moisture content of the macaroni is gradually reduced to 12.5% on the wet basis (14.3% dry basis). This period requires the longest drying time and the most careful control of the drying conditions. Numerous drying schedules are in use, some of which are described by Hoskins (11). Binnington and Geddes (7) described a procedure for experimentally drying macaroni using a constant temperature and a gradually decreasing humidity. Commercial driers are discussed by Hoskins.[4] Improper control of drying leads either to cracks and checking or to mold formation.

The objective of the present research was to determine the factors in drying which affect the physical properties of macaroni with particular reference to checking and cracking. The coefficient of thermal expansion, the coefficient of moisture shrinkage, and the modulus of rupture were determined and the data used to compute stresses in the macaroni due to temperature and moisture gradients.

Determination of Physical Constants of Macaroni Products

Thermal Expansion. Length changes were measured by a quartz tube dilatometer patterned after that described by the American Society for Testing Materials (2). The outer tube had a length of 20 inches and an inside diameter of $\frac{5}{16}$ inch. The inner rod had a length of $11\frac{5}{8}$ inches and a diameter of $\frac{3}{16}$ inch. A Federal dial gauge graduated to 0.0002 inch per division was bolted to a bracket on the quartz tube, the stem of the dial gauge being concentric with the quartz tube.

Samples of commercial and experimentally processed macaroni were cut to a length of 3.4 inches with ends perpendicular to the axis of the tube. The length of the conditioned test specimen was measured at room temperature with a vernier micrometer to the nearest 0.001 inch. The test specimen was mounted in a dilatometer placed in a bath maintained at 30.1°C. for 10 minutes, a sufficient time for the macaroni to reach bath temperature. The reading of the dial gauge was recorded. The dilatometer was carefully transferred to a second bath at 3.7°C. or 67.8°C. and the dial gauge read after 10 minutes.

[4] Private communication.

The dilatometer was returned to the first bath and the procedure repeated.

The average coefficient of linear thermal expansion was calculated by the formula

$$L = L_o[1 + \alpha(T - T_o)]$$

where α = the coefficient of linear thermal expansion inches per inch of length per °C.
$(L - L_o)$ = the average of the changes in length due to heating and cooling.
L = the length at temperature T.
L_o = the length of the test specimen at temperature T_o.
$(T - T_o)$ = the temperature difference in °C. over which the changes in length are measured.

The linear coefficients of thermal expansion which were found are presented in Table I.

TABLE I

COEFFICIENT OF THERMAL EXPANSION OF MACARONI

Source	Protein[1]	Moisture	Temperature range	Inches per inch of length per °C.
	%	%	°C.	α
Commercial	11.8	11.1	20–80	54×10^{-6}
Experimental, Stewart wheat	11.8	8.6	3.7–30.1	55×10^{-6}
Experimental, Stewart wheat	11.8	8.6	30.1–67.8	64×10^{-6}

[1] 14.0% moisture basis.

Coefficient of Moisture Shrinkage. Six pieces of commercial macaroni, protein 10.7% (14.0% moisture basis), containing 31.5% moisture (dry basis), which had been made on a conventional hydraulic press subjected to a two-hour preliminary drying with air having a 7°F. wet bulb depression, were rapidly cut with a razor blade to a length of 1.9 inches. All were marked with the light impression of a pair of dividers set at 2.530 cm. The samples, kept in a glass weighing bottle, were weighed and then placed successively in desiccators filled with sulfuric acid of the proper concentration to maintain 80, 60, 40, and 20% relative humidity. A vacuum oven was used to obtain a dry sample. The distance between the gauge marks was measured every two or three days with a Gaertner traveling microscope capable of being read to 0.0001 cm. The samples were then placed in the desiccator of lower relative humidity and the process repeated.

The coefficient of linear shrinkage due to changes in moisture

content was calculated by the formula

$$L = L_o[1 + \alpha'(W - W_o)]$$

where α' = the coefficient of linear shrinkage inches per inch of length per unit percentage decrease in moisture content (on a dry basis).

L = the length at the moisture content W.

L_o = the length at the moisture content W_o.

$(W - W_o)$ = the difference in moisture content per cent on a dry basis.

The values of α', coefficient of linear shrinkage due to changes in moisture content for long macaroni, decreased with an increase in moisture content, having a value of 0.003 inch per inch of length per per cent change in moisture at 7% moisture, 0.0025 at 19% moisture, and 0.0017 at 26% moisture. These moisture values are all expressed on a dry basis.

Modulus of Rupture. The flexural properties of macaroni products were determined using a modification of the Flexural Test of Plastics described by the American Society for Testing Materials (1). The breaking loads for spaghetti were too small for available testing machines. A torsion balance (capacity 1 kg.) was equipped with a constant-level water-feed and receiver. The water-feed rate was adjusted to give the desired rate of loading. The water supply was cut off by a momentary contact switch on the balance which operated a manual reset relay which shut off the water supply and stopped an electric timer. A sample support rested on the other pan of the balance, above the center of which was a fixed arm for center loading of the specimen. An optical system comprised of a galvanometer lamp, a 90° prism, a lightweight mirror mounted on the central pointer of the balance, and a chart supported on the wall of the adjacent room was used to obtain deflection data during the flexure test. The deflection measuring system was calibrated by recording the deflection indicated by known displacements produced by a screw micrometer mounted in contact with the specimen plate. The loading nose and plate supports were cold-rolled steel 0.25 inch in diameter. A span of 2 inches was used for the tests on spaghetti and macaroni, while a span of 0.5 inch was used for egg noodles. Samples of commercial spaghetti made on a conventional hydraulic press were dried to between 26 and 10.9% (dry basis) in air-conditioned dryers, placed in closed containers, and tested as rapidly as possible to minimize any surface drying effects.

The modulus of rupture (flexural strength) was calculated from the load required to break the specimen, tested as a simple beam loaded at midspan, by the following formulas:

a. For a solid cylinder of circular cross section

$$S = \frac{8PL}{\pi D^3}$$

b. For a hollow cylinder of circular cross section

$$S = \frac{8PLD}{\pi(D^4 - d^4)}$$

c. For a beam of rectangular cross section

$$S = \frac{3PL}{2bd^2}$$

where S = modulus of rupture, pounds per square inch
P = breaking load, pounds
L = distance between supports, inches
D = outside diameter of beam tested, inches
d = inside diameter of beam tested, inches, or
b = width of rectangular beam tested, inches.

As shown in Fig. 1, the modulus of rupture of samples of spaghetti, protein 12.3% (14.0% moisture basis), taken at varying stages in the drying process increases as the material becomes drier. The maximum strength for these samples was 2,270 lb. per square inch for a moisture content of 7% (dry basis). It is possible that these samples were left with a high moisture gradient when the final drying was started; this would lead to large permanent stresses which would account for these lower values. Also shown in Fig. 1 is an estimated curve for commercial spaghetti using the value of 5,400 lb. per square inch at 11% moisture (dry basis).

The importance of a relatively high modulus of rupture in determining the amount of broken macaroni found on the bottom of the drying racks has been brought out by Winston and Jacobs (23). The improper control of the drying operation has much larger effects on the breakage than does the addition of lecithin. A macaroni which is full of checks must be sold as feed or if clean is sometimes reground and reprocessed.

The breaking strengths of commercial and laboratory processed macaroni have been presented by Binnington and Geddes (7) in a form which permits the calculation of the modulus of rupture. Commercial macaroni had a modulus of rupture lying between 6,100 and 7,300 lb. per square inch. Laboratory-processed material had a modulus of rupture lying between 3,600 and 5,200 lb. per square inch.

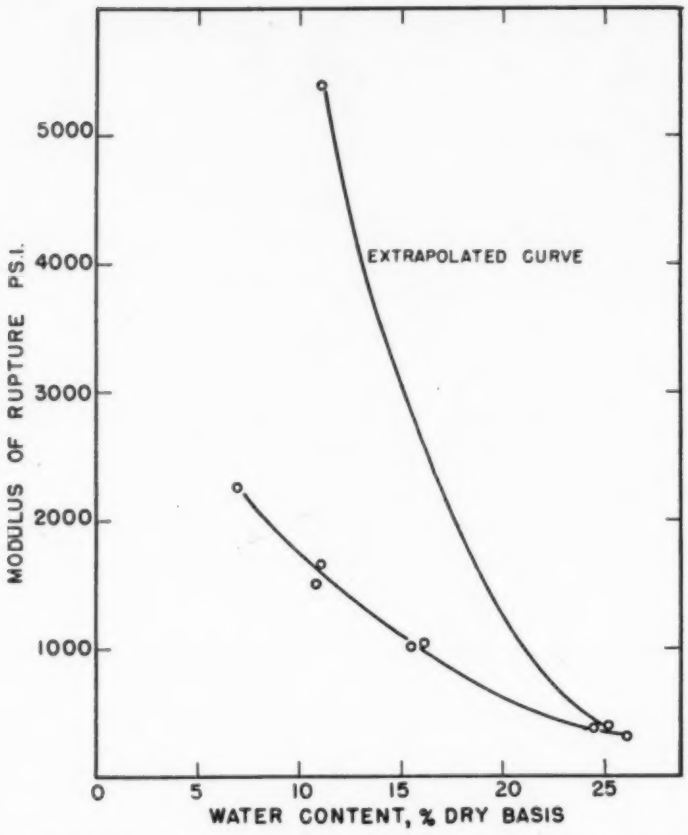

Fig. 1. Modulus of rupture of spaghetti as a function of water content. The extrapolated curve is an estimated one for commercial spaghetti using a value of 5,400 lb. per square inch for the modulus of rupture at 11% moisture (dry basis).

The modulus of rupture has been determined for four samples of commercial spaghetti, protein 11.5% (14.0% on moisture basis), having a moisture content of 11% (dry basis). The average of the 10 readings from each sample was used to compute the modulus of rupture presented in Table II.

TABLE II

MODULUS OF RUPTURE OF COMMERCIAL SPAGHETTI

Diameter *in.*	Modulus of rupture *lb./sq. in.*
0.064	5,120
0.073	5,770
0.085	5,500
0.089	5,340
Average	5,430

Binnington, Johannson, and Geddes (8) have reported the relation between protein content of wheat and the breaking strength of macaroni. Their data reported in arbitrary units have been converted for purposes of comparison into physical units, modulus of rupture in pounds per square inch, in Table III. It was necessary to assume that the distance between supports in their flexural test was 1 inch.

Winston and Jacobs (23) have reported the breaking strength of commercially dried spaghetti processed with and without added lecithin. The sample was supported on glass rods 6 inches apart and weights were added at the center until the breaking point of the spaghetti was reached.

TABLE III

EFFECT OF PROTEIN LEVEL ON THE MODULUS OF RUPTURE

Protein content[1]	Mean breaking strength	Modulus of rupture
%	*Units*	*lb./sq. in.*
10.5	153.0	3,978
11.3	166.2	4,321
12.3	169.5	4,407
13.2	190.4	4,950
13.9	196.8	5,117

[1] 14.0% moisture basis.

Assuming that the diameter of the spaghetti was 0.080 inch, the modulus of rupture, computed from their data is 4,333 lb. for the control and 4,240 lb. for the spaghetti to which 0.5% lecithin had been added. The addition of 0.5% lecithin does not have an appreciable influence upon the breaking strength of spaghetti in comparison with the larger effects attributable to drying conditions. Winston and Jacobs provide data showing that the use of lecithin increases the amount of broken pieces from 0.28% for the control to 0.34% with the added lecithin.

Young's Modulus of Elasticity. Modulus of elasticity may be defined as the ratio of the internal stress to the strain and is a number characteristic of the material at a given moisture content. The procedure already described for determining the modulus of rupture was followed in measuring the modulus of elasticity. The deflections were measured at 5-second intervals during the loading of the sample.

The modulus of elasticity (Young's Modulus) of spaghetti determined as a simple beam loaded at midspan was calculated from the theory of flexure using the elastic curve of a beam, with the formula

$$E_b = \frac{4L^2P}{3\pi D^4}$$

$$= \frac{4L^2(P70 - P20)}{3\pi D^4(\Delta 70 - \Delta 20)}$$

where E_b = modulus of elasticity
L = distance between supports, inches
P = load, pounds
Δ = deflection at center, inches
$P70, 70$ = load and deflection at 70% of maximum load
$P20, 20$ = load and deflection at 20% of maximum load
D = diameter of spaghetti, inches.

The results are presented in Fig. 2 as modulus of elasticity versus the moisture content expressed on a dry basis.

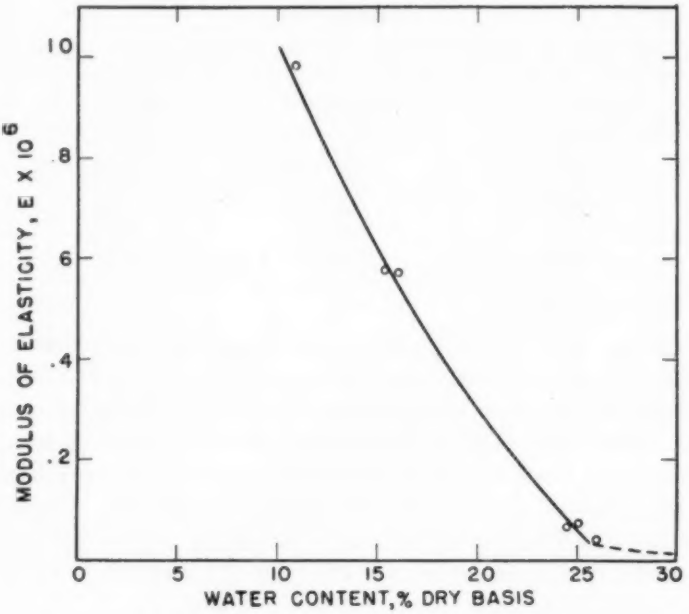

Fig. 2. Modulus of elasticity of spaghetti as a function of water content.

The modulus of elasticity rises rapidly as the moisture content is decreased below 26% and since the equipment used was not suitable for wetter material, data on modulus of elasticity of flour dough reported in the literature (16, 17, 18) have been used to extrapolate the curve to 30% water (dry basis).

Moisture Gradients in Drying

A sample of macaroni dough, protein 11.9% (14.0% moisture basis), was rolled to a thickness of 0.075 inch. Five layers were placed in an aluminum moisture dish and drying proceeded from only one face. The sample was placed in the experimental dryer described

by Ritchell *et al.* (15) and dried for 4 hours. The dry bulb was held at 35°C., relative humidity at 72%, and the air velocity at 640 feet per minute. Upon removal from the dryer, the outer layer was split into two portions, placed in aluminum moisture dishes, and analyzed for moisture by the vacuum oven method at 99°–100°C. The rate of drying below the surface was very slow. The data were plotted and from the curve it could be estimated that the level at which there had been no moisture change was in the top layer.

A sample of elbow macaroni from a Buhler Continuous press, protein 11.9% (14.0% on moisture basis), was rolled to a thickness of 0.014 inch. Seven layers were made into a sandwich and placed in the same dryer for 2 hours at 95.4°F. with a relative humidity of 70.5% and an air velocity of 670 feet per minute. The equilibrium moisture corresponding to this humidity is 16% (dry basis). The moisture content dropped from 46% to an average of 28% in 2 hours. The distribution of water obtained from the analysis of the delaminated sandwich is presented in Fig. 3. A difference of 18% existed between the center and the outside surface. The conditions of this test were similar to those which have been used in several commercial plants. In the rest period, "sweating," the surface would be expected to rise to some point between 26 and 30% either by addition of water from the air or by further diffusion of water from the center toward the surface. Plant tests have shown that a truck containing 175 lb. of wet macaroni in a period of rest often gains up to 2% water on a dry basis. This would indicate that water is added to the surface in addition to a diffusion of water from the interior.

A sample of macaroni dough handled in a manner similar to the previous tests was dried for 8 hours under the following conditions: dry bulb, 90.8°F.; wet bulb difference, 25.7°F.; relative humidity, 23%; equilibrium moisture content, 7.7% (dry basis); air velocity, 670 feet per minute. At the end of drying a difference of 11% in moisture content existed between the center and the surface. The conditions of this test are far more severe than can be tolerated in commercial practice if the product is to be in one piece free from large cracks. This test indicates that a commercial spaghetti of 0.062 inch radius could drop from the moisture content it contains when pressed (44–46%) to 19% in 8 hours. However, the gradient in the early stages of drying must have exceeded 25% moisture (dry basis) and this is more than the material can withstand without checking.

Samples of elbow macaroni, protein 11.9% (14.0% moisture), were obtained from a Buhler Continuous press in a local plant. The dough which passed through the die has been assumed to have a diffusivity similar to the extruded material when rolled out between steel rollers.

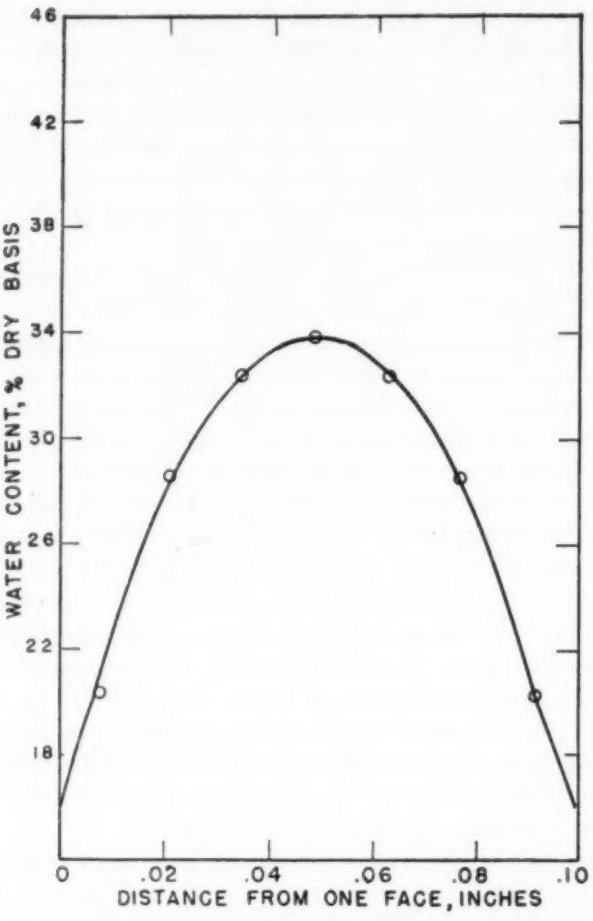

Fig. 3. Moisture gradients in preliminary drying. The data were obtained by drying a sandwich of seven sheets, each 0.014 inch thick, for 2 hours at 95.4°F. with a relative humidity of 70.5% and an air velocity of 670 feet per minute, and determining the moisture content in the various layers of the delaminated sandwich.

The dough was rolled out to a thickness of 0.02 to 0.03 inch and folded to make a sandwich of seven layers, approximately 2 by 5 inches. The four edges were pressed together by a $\frac{1}{16}$ inch wide straightedge to prevent delamination during drying. Seven similar samples were placed in a horizontal position supported at the edges by a thin wire framework 2 inches above the floor of a controlled humidity dryer described by Ritchell, Piret, and Mann (15). The average air velocity used in this drying experiment was 637 feet per minute; the dry bulb temperature was 35°C. The humidity was varied during the experiment to approximate a commercial schedule for a 30-hour drying period. At stated periods of time one of the seven similar samples

was removed, the edges trimmed back to eliminate those portions where drying was occurring also through the edges, and carefully delaminated with a razor. Each layer was placed in an aluminum moisture dish with a tight-fitting cover and the moisture content was determined by drying in a vacuum oven at 100°C. for 48 hours without grinding the sample. An attempt was made to simulate the commercial methods by 1 hour of rapid drying at a low relative humidity (80%) followed by a rest period of 1 hour in air of high relative humidity (93%) with the fan turned off. The fan was then turned on and the drying was continued.

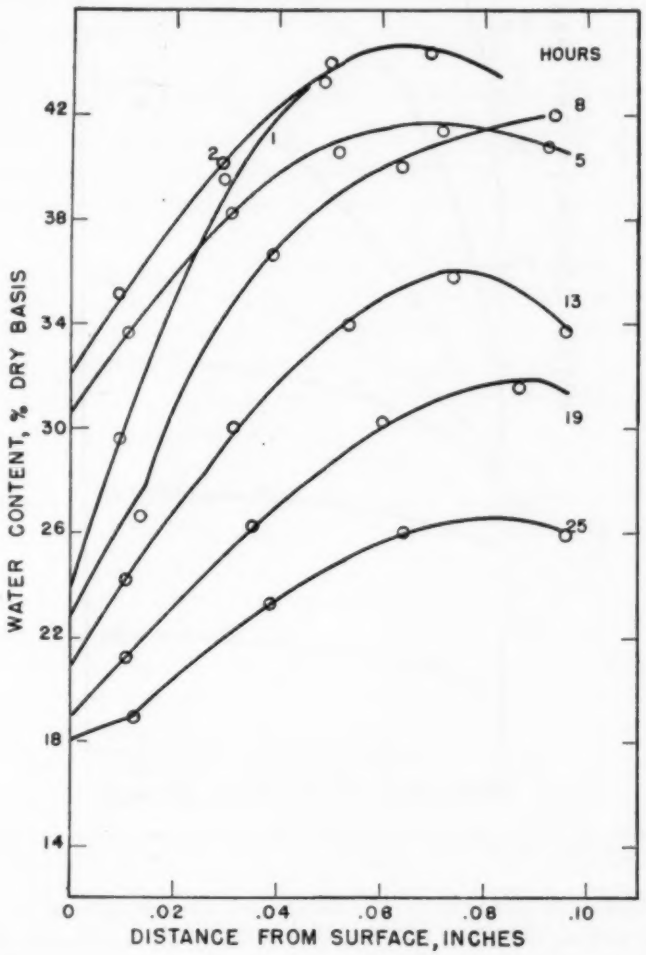

Fig. 4. Moisture gradients in drying macaroni at 35°C. for different times. The data were obtained by drying seven sheets of macaroni dough, each 0.02 to 0.03 inch thick, made into sandwiches at 35°C., with the relative humidity varied to approximate a commercial 30-hour drying schedule. At the stated time intervals one sandwich was removed, the layers separated, and their moisture contents determined.

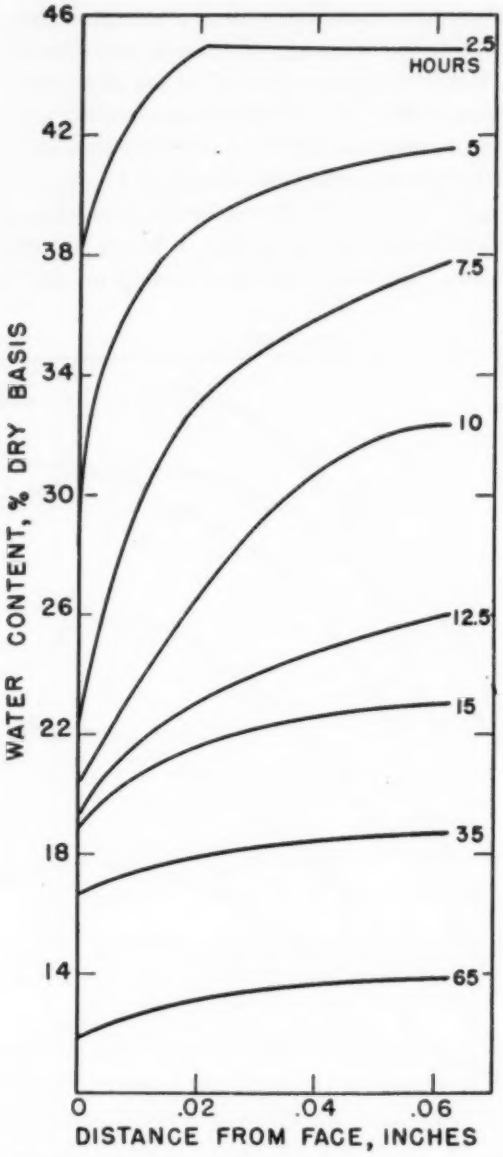

Fig. 5. Moisture gradients in drying macaroni for different times. The curves are based on data presented by Binnington and Geddes (7) for the laboratory drying of macaroni.

The moisture content of the macaroni as a function of distance below the drying surface is plotted in Fig. 4 for drying times of 1, 2, 5, 8 ,13, 19, and 25 hours. The preliminary drying for 1 hour at a relative humidity of 80% established a moisture difference of 22% (dry basis) between the surface and the center of the slab. The

effect of the sweating was to add water to the surface, raising the surface moisture content from 21 to 30%; this had the over-all effect of reducing the moisture gradient in the slab from 22 to 13% (dry basis). The moisture content of the surface was gradually decreased by lowering the relative humidity and this resulted in a gradient between the center and the outside of 13% after 13 hours. After 25 hours the moisture gradient was still 8.5%.

The moisture distribution has been approximated in Fig. 5 from the data presented by Binnington and Geddes (7) for the laboratory drying of macaroni. The gradients in the early stages of drying are large in this test because of the short preliminary drying which was given the macaroni.

Discussion of Factors Causing the Checking of Macaroni

Semolina when mixed with water first forms a crumbly mass which is then kneaded into a dough and extruded by hydraulic or continuous-type presses. The dough in this form is plastic but it gradually loses this property during drying and behaves more nearly like a brittle material, that is, a material which is weakest in tension. Macaroni in the various stages of its drying is initially ductile (i.e., capable of being permanently pulled out), then it is both ductile and elastic in the region where viscous flow relieves stress, and is finally brittle at lower moisture contents. There is no sharp dividing line in which the properties suddenly change from one type to another. The location of a region over which viscous flow can occur is not sharply defined at this time although many plant operators find that one-third of the water in the dough can be removed rapidly and still produce a satisfactory product. The modulus of elasticity curve may be extrapolated to zero at a moisture content between 26 and 27% (dry basis). However, if the data from the literature for a bread dough are used to extend this curve, a sharp change in the slope occurs in this same moisture range.

Large samples of elbow macaroni taken from a commercial short-goods dryer were stored in closed cans for a period of 1.0 and 1.5 hours. The samples were examined by a manufacturer of macaroni drying equipment and by the authors. The lower limit for satisfactory remoistening was considered to be between 26.5% for an airtight remoistening chamber and 30% (dry basis) for a remoistening chamber in which there was a lower relative humidity. The amount of bound water computed from data published by Swanson (19) was found to be 28.2% (dry basis). Vail and Bailey (22) found that the bound water was 28.6% (dry basis) at freezing temperatures, while Baker, Parker, and Mize (5) calculated the bound water to be 27.3% (dry basis).

The data indicate that the limiting moisture content permitting viscous flow lies between 26 and 30% (dry basis). This must be further qualified by a consideration of the strength of brittle solids. At low temperatures a point is often reached called the brittle point. This probably represents the temperature at which both elastic modulus and plastic viscosity become so high that sufficient deformation cannot take place rapidly enough to prevent the stresses imposed in a short-time test from exceeding the strength of the material.

A brittle material shows elastic properties including not only the instantaneous response but also lesser known reactions, such as primary creep, elastic after-working, and delayed elastic effect. The strength of a brittle material depends on such factors as time, chemical environment, and prestressing or permanent stresses which result from the methods of formation and drying. Recent theory applied to brittle materials emphasizes that various molecular or atomic mechanisms are the essential factors which determine the time for fracture. Since the elementary process of rupture consists of the irreversible separation of the atoms or molecules, no other external force except tension can bring about this separation. Failure occurs at the point having the largest tensile stress. Prestressing of the surface under compression increases the tensile strength by making surface flaws less important. Poncelet (13) states that it is unnecessary to resort to any hypothesis of the preexistence of flaws to explain the behavior of homogenous brittle solids under stress; the application of mechanics and elasticity to the particulate structure of matter and of the electrostatic character of the particle appears to suffice. A fracture in a brittle solid begins at or near the most positive principal stress, and once started tends to propagate further. The condition of the outside surface, the atmosphere in direct contact with the solid, and the duration of the stress all have an effect on the value of the strength found for a brittle solid. The tensile strength of identical solids always shows a scattering of values instead of identical reproducible values which are characteristic of common ductile materials. Binnington, Johannson, and Geddes (8) found a large scattering of their results for the flexural strength of macaroni; which was not due to the differences in dimensions. The theory of the failure of brittle solids provides an explanation of their observation.

The stresses that originate in a tubular strand of macaroni or spaghetti which is subjected to a moisture gradient may be treated by the equations developed for stresses which originate from a change in tempering (6, 20, 21). The moisture gradient is quite similar in shape to the thermal gradient that has been used in the derivation of the

thermal stress equations. Moisture stresses may arise from one of the following causes: (1) an increase in moisture content at the surface, the moisture content of the sample having been uniform; (2) a decrease in moisture content at the surface, the moisture content of the sample having been uniform; and (3) the removal of water from a wet dough.

Increase in Moisture Content of the Surface. A solid tube or cylinder that has a uniform moisture distribution placed in an air stream of the same temperature, but whose relative humidity is greater than that corresponding to the equilibrium value for the moisture in the macaroni or spaghetti, will pick up water from the air. As water is added to the surface of the macaroni the outside of the tube tends to expand more than the inner layers. The outside of the cylinder is not free to expand in the normal manner and is therefore under compression; at the same time the inner layers will be stretched by the outer layers and will be in tension. The longitudinal and tangential stresses change continuously from compression at the surface to tension in the interior and necessarily pass through an intermediate zone of zero stress. The stresses formed are tabulated below:

DIRECTION OF STRESS	LOCATION	TYPE
Tangential stress	Inside radius	Tension
Tangential stress	Outside radius	Compression
Longitudinal stress	Inside radius	Tension
Longitudinal stress	Outside radius	Compression
Radial stress	All radii	Tension

As long as the outside layer is in compression, the macaroni will be stronger and will be less likely to check on the outside surface, but still may fail by checking in the interior due to the tensile stress at the inside radius. If water is added until the moisture distribution is again uniform at the new level corresponding to the relative humidity of the air surrounding the macaroni, the stresses caused by this moisture gradient will have disappeared with that gradient. These stresses may be called temporary since they continue only as long as the moisture gradient is maintained.

Decrease in Moisture Content of the Surface. A solid tube or cylinder which has a uniform moisture distribution when placed in an air stream of the same temperature but of a lower relative humidity than corresponds to the equilibrium value for the moisture in the macaroni will lose water to the air. This establishes a moisture gradient in the sample. Temporary stresses will be developed as shown in the table below:

DIRECTION OF STRESS	LOCATION	TYPE
Tangential stress	Inside radius	Compression
Tangential stress	Outside radius	Tension
Longitudinal stress	Inside radius	Compression
Longitudinal stress	Outside radius	Tension
Radial stress	All radii	Compression

The macaroni will be weakest when the outside surface is in tension and failure will be most likely to occur at the outer surface. When equilibrium is reached at the lower moisture level corresponding to the new relative humidity, the temporary stresses caused by the moisture gradient will have disappeared.

Removal of Water from a Wet Dough. The removal of water from macaroni causes the formation of moisture gradients as shown in Figs. 3, 4, and 5. The use of controlled relative humidity schedules or periods of alternate drying and resting have been used to minimize the magnitude of these gradients.

The stresses established by drying may be considered under the two following cases: (1) Macaroni with a uniform moisture gradient exists in the stress-free condition when it passes the lower limit of moisture content where stresses can be relieved by viscous flow; (2) A moisture gradient exists within the stress-free macaroni when it passes the lower limit of moisture content where stresses can be relieved by viscous flow.

Stress-free Macaroni, No Moisture Gradient. Drying will put the macaroni under a temporary stress and so long as the macaroni is not checked at any point due to an excessive gradient, the strength will be regained when the moisture distribution again becomes uniform. There will be no permanent stresses in this macaroni. The strength of this macaroni would be greater if the surface were under a slight compression, for this would minimize the effect of the surface irregularities as stress concentrators.

Stress-free Macaroni, Moisture Gradient Present. In this case a moisture gradient exists in a material that is free from stress such as might well exist in macaroni where plastic flow has occurred to release the stresses formed in the early stages of drying. The removal of this gradient will cause stresses opposite in sign but equal in magnitude to the stresses which would be caused by the same moisture gradient in a sample initially free of stress. The removal of such moisture gradients will cause stresses the same as shown above.

These stresses are called permanent stresses and can be removed permanently only by an annealing process. It has been possible to remove permanent stresses in the cases of certain thermoplastics by the addition of radiant heat to raise the temperature of the plastic

above the point at which internal stresses are relieved. This is not considered practical for macaroni for several reasons. Macaroni tends to decompose before any softening point is reached, color would be injured at higher temperatures, and there would be the cost and difficulty of cooling the macaroni without checking it due to excessive changes in moisture content at the surface.

The customary preliminary drying followed by a remoistening or sweating to obtain a uniform moisture distribution tends to produce a stress-free macaroni with a moisture gradient. A portion of the stresses are released by viscous flow during the remoistening period. If moisture equilibrium was not obtained and drying was started again, it would be possible to leave the region where internal stresses can be relieved with a large moisture gradient but with relatively stress-free macaroni. In this case, no matter how much care is given in drying, the final product will be weak due to the permanent stresses and may even be checked on the inside as the moisture gradient is reduced at the end of the drying period.

A like case would result from a short preliminary drying with remoistening at a high moisture level, followed by a resumption of drying establishing a new moisture gradient by the time the material has reached the lower limit for plastic flow. Where this moisture gradient is large the macaroni may fail by tension at the inner surface upon the removal of the moisture gradient at the end of drying. This type of failure is one of the most puzzling to commercial operators, for the macaroni looks and feels strong as long as the drying is in progress. Soon after the fans are stopped the cracking starts.

It is not likely that there would be a sudden change in properties above a certain moisture content. It is more likely that when there is some free water present (above about 26% on a dry basis), some viscous flow can occur. This releases a portion of the stresses permitting a macaroni with a moisture gradient to exist in a stress condition less than would be shown by computation. Thus one would not expect to attain a stress-free condition when dry. A strong product would result at the end of drying if one passed through this region with a small moisture gradient. This macaroni is, then, more susceptible to surface cracking in the earlier stages of drying than when the permanent stresses place the outer surface in compression.

The equations of Barker for the thermal stresses in a long tube of circular cross section may be modified for the computation of the maximum tangential stress due to the formation of a moisture gradient between the inner and outer surface of the tube. The modified equations in which moisture, W, is substituted for temperature, T, and the moisture coefficient of shrinkage, a', is substituted for the

thermal coefficient of expansion, a, are as follows:

$$P_r = \frac{(W_1 - W_2)\frac{1}{\gamma} E\alpha}{2\left(\frac{1}{\gamma} - 1\right)(r_2^2 - r_1^2)\ln\frac{r_1}{r_2}} \left[\frac{r_1^2 r_2^2}{r^2}\ln\frac{r_1}{r_2} + r_2^2 \ln r_2 - r_1^2 \ln r_1 - (r_2^2 - r_1^2)\ln r\right]$$

$$P_\theta = \frac{(W_1 - W_2)\frac{1}{\gamma} E\alpha}{2\left(\frac{1}{\gamma} - 1\right)(r_2^2 - r_1^2)\ln\frac{r_1}{r_2}} \left[\frac{r_1^2 r_2^2}{r^2}\ln\frac{r_2}{r_1} - (r_2^2 - r_1^2)(\ln r + 1) + r_2^2 \ln r_2 - r_1^2 \ln r_1\right]$$

$$P_z = \frac{(W_1 - W_2)\frac{1}{\gamma} E\alpha}{2\left(\frac{1}{\gamma} - 1\right)(r_2^2 - r_1^2)\ln\frac{r_1}{r_2}} \left[2\, r_2^2 \ln\frac{r_2}{r} - r_1^2 \ln\frac{r_1}{r} - (r_2^2 - r_1^2)\right]$$

where $W_1 - W_2$ = difference in water content between the inside and outside of the tube, per cent on a dry basis
T = temperature of the tube, °C.
r = radius of the tube, inches
γ = Poisson's ratio for the material
E = modulus of elasticity, Young's
a' = moisture coefficient of shrinkage of the material
P_θ = tangential tensile stress
P_r = radial tensile stress
P_z = longitudinal tensile stress
Suffixes 1 and 2 refer to the inside and outside of the tube, respectively.

A solution for a typical case where the ratio of r_2/r_1 is 1.7 gives the maximum tangential stress produced by the formation of a moisture gradient of 1% to be 1,800 lb. per square inch.

The theoretical moisture gradient which will not cause checking has been computed for the two modulus of rupture curves of Fig. 1. Using the experimental data, the macaroni will withstand a moisture gradient of from 0.75 to 1.5% without fracture, depending on the ratio of r_2/r_1 and the moisture content. From the extrapolated data on the commercial product, the gradient varies from 1.5 to 2.5%. A thin-walled product can withstand a greater stress than can a thick-

walled product. These results merely indicate trends since the failure in brittle solids depends on many factors, including the magnitude of the initial stress distribution. It is entirely possible that the allowable tensile stress of stress-free macaroni may be several times greater than has been determined.

The effect of temperature alone can be calculated for a hypothetical case by assuming that macaroni containing 12% moisture (dry basis) at 90°F. is suddenly placed in a current of moving air at 70°F. with a relative humidity such that no changes occur in the surface moisture content. The maximum tangential stress calculated for these conditions is 19 lb. per square inch which is a very small value in comparison with the allowable value of 2,000 to 5,000 lb. per square inch for the modulus of rupture.

Observation of relative humidities on various floors of commercial plants has shown that relative humidity variations from 65 to 50% are not uncommon. Variations from 70 to 30% have been measured in the winter on numerous occasions.

Macaroni in equilibrium with air of 65% relative humidity would have a moisture content of 14.9% (dry basis), while after attaining equilibrium in air of 50% relative humidity, the moisture content would be 12.3% so that the moisture gradient between the surface and the interior would be 2.6%. The maximum tangential stress due to a difference of 1% was calculated to be 1,800 lb. per square inch. For a difference of 2.6% the maximum tangential stress would be 4,700 lb. per square inch. This would not cause checking of a strong macaroni.

LeClerc (12) has stated that macaroni should not be subjected to sudden changes in temperature as this may cause the product to warp. A study of the relative magnitude of the stresses due to temperature and relative humidity changes shows clearly that it is the moisture content of the air and not the temperature that is responsible for the checking of macaroni products. Probably the origin of this line of thought in the industry lies in the fact that a plant having widely different temperatures will also have large differences in the relative humidity.

A second cause of checking is the disappearance of the moisture gradient that existed in the macaroni when it was in the plastic state in a stress-free condition.

Literature Cited

1. American Society for Testing Materials. Tentative method of flexural test of plastics (D 790–45T). 1946 Book of ASTM Standards, Part III-B: Philadelphia (1946).
2. American Society for Testing Materials. Standard method of test for

coefficient of linear thermal expansion of plastics (D 696–44). 1946 Book of ASTM Standards, Part III-B: Philadelphia (1946).
3. ANONYMOUS. Cuts production time 45 per cent; eliminates spoilage 100 per cent. Food Ind. **8**: 330–331 (1936).
4. BAILEY, C. H. Physical tests of flour quality. Wheat Studies Food Research Inst. **16**: 243–300 (1939–40).
5. BAKER, J. C., PARKER, H. K., and MIZE, M. D. The distribution of water in dough. Cereal Chem. **23**: 30–38 (1946).
6. BARKER, L. H. Calculation of temperature stresses in tubes. Engineering **124**: 443–444 (1927).
7. BINNINGTON, D. S., and GEDDES, W. F. Experimental durum milling and macaroni making technique. Cereal Chem. **13**: 497–521 (1936).
8. BINNINGTON, D. S., JOHANNSON, H., and GEDDES, W. F. Quantitative methods for evaluating the quality of macaroni products. Cereal Chem. **16**: 149–167 (1939).
9. EARLE, P. L., and ROGERS, M. C. Drying macaroni. Ind. Eng. Chem. **33**: 642–647 (1941).
10. HOPKINS, I. L. A useful convention for calculating the modulus of elasticity and a nomogram to help in computing the elastic modulus and the flexural strength. ASTM Bull. **98**: 29–30 (1939).
11. HOSKINS, G. G. Science dispels mystery from macaroni drying. Food Ind. **4**: 48–51 (1932).
12. LECLERC, J. A. Macaroni products. Cereal Chem. **10**: 383–419 (1933).
13. PONCELET, E. F. Nature of strength and failure in brittle solids. *In* Colloid chemistry, theoretical and applied, Vol. 6, ed. by Jerome Alexander. Reinhold: New York (1946).
14. RENAUDIN, C. La fabrication industrielle des pâtes alimentaires. Dunod: Paris (1946).
15. RITCHELL, E. C., PIRET, E. L., and MANN, C. A. Laboratory apparatus for controlled experimental drying of foods. Proc. Inst. Food Technol. **1941**: 23–28 (1941).
16. SCHOFIELD, R. K., and SCOTT-BLAIR, G. W. Relationship between viscosity, elasticity and plastic strength of soft materials as illustrated by some mechanical properties of flour doughs. I. Proc. Roy. Soc. (London) **138A**: 707–718 (1932).
17. SCHOFIELD, R. K., and SCOTT-BLAIR, G. W. Relationship between viscosity, elasticity and plastic strength of soft materials as illustrated by some mechanical properties of flour doughs. II. Proc. Roy. Soc. (London) **139A**: 557–566 (1933).
18. SCHOFIELD, R. K., and SCOTT-BLAIR, G. W. Relationship between viscosity, elasticity and plastic strength of soft materials as illustrated by some mechanical properties of flour doughs. III. Proc. Roy. Soc. (London) **141A**: 72–85 (1933).
19. SWANSON, C. O. Physical properties of dough. Burgess: Minneapolis (1943).
20. TIMOSHENKO, S. Theory of elasticity. McGraw-Hill: New York (1934).
21. TIMOSHENKO, S. Strength of materials. Pt. II. Advanced theory and problems. 2nd ed. Van Nostrand: New York (1941).
22. VAIL, GLADYS, and BAILEY, C. H. The state of water in colloidal gels: Free and bound water in bread doughs. Cereal Chem. **17**: 397–417 (1940).
23. WINSTON, J. J., and JACOBS, B. R. Using soybean lecithin in the macaroni industry. Food Ind. **19**: 166–169 (1947).

EFFECT OF HEAT TREATMENT ON THE SULFHYDRYL GROUPS OF MILK SERUM PROTEINS [1]

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ABSTRACT

When milk serum protein sols containing 5.0 to 6.0 g. of protein per liter in phosphate buffer at pH 6.6 were heat-treated under nitrogen, dialyzed, and stored in air, the titratable —SH groups expressed as cysteine and determined by the o-iodosobenzoate procedure decreased from 0.87% for the control to 0.68, 0.51, and 0.42% for sols maintained at 70°, 75°, and 80°C. respectively for 30 minutes. These decreases could not be accounted for by the small quantities of volatile sulfur which were evolved nor by any change in the cysteine or methionine values. Failure to detect cystine in hydrolyzates of either unheated or heated samples indicates that the lower —SH titer of the heat-treated samples was due to aggregation of the protein molecules rather than to oxidation of sulfhydryl to disulfide linkages.

When milk serum protein sols were heat-treated in air and nitrogen respectively and titrated immediately upon cooling, a decrease in —SH groups available to o-iodosobenzoate only occurred in air. Both thiamine disulfide and ferricyanide gave very low titers for unheated milk serum protein, the —SH values expressed as cysteine being 0.02 and 0.09% respectively as compared with 0.63% by titration with o-iodosobenzoate. However, heat treatment increased the groups available to these reagents.

The possibilities of heat-induced interactions between the several electrophoretically different components of the milk serum and of the protein sulfhydryls with lactose are discussed.

Since sulfhydryl compounds cause softening of dough and depression of loaf volume, the decrease in titratable —SH groups as measured by titration with o-iodosobenzoate serves to explain the improvement in the baking value of nonfat milk solids that can be effected by the proper heat-treatment of separated milk before drying.

It is well known that the detrimental effect of nonfat dry milk solids on the consistency of dough and the loaf volume of bread can be overcome by heating the separated milk before drying (11, 12, 23, 28). Furthermore, the serum protein fraction of the milk has been shown to be responsible for both the dough softening (23, 30) and the loaf volume depression (15, 23).

The fundamental reason for the dough-softening action of the milk serum proteins has not been elucidated satisfactorily. However, the

[1] Manuscript received April 8, 1949. Presented at the Annual Meeting, May, 1949.
Paper No. 2468, Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper represent a portion of a thesis presented to the Graduate School of the University of Minnesota by R. A. Larsen in partial fulfillment of the requirements for the Ph.D. degree, June, 1947.
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similarity between this phenomenon and the dough-softening effect of reducing substances (see Shen and Geddes, 27) would lead one to suspect that such powerful reducing groups in the protein as the sulfhydryls might be involved. This suggestion actually was made by Stamberg and Bailey (30), who found that while fresh skimmilk exhibited a polarographic wave characteristic of sulfhydryl compounds, such a wave could not be demonstrated with heated skimmilk. At first glance, this result appears to be contradictory to the generally accepted idea that denaturation of proteins by heat or other means liberates sulfhydryl groups (1, 26). It also seems contrary to the established fact that heat-treatment of milk causes the appearance of a positive nitroprusside test (13, 19) and of substances that reduce thiamine disulfide (14). Stamberg and Bailey (30) suggested that this difficulty might be resolved by considering that heat causes a reorganization of the protein micelles in such a way that the over-all activity of the sulfhydryl groups is decreased, while at the same time, some of the —SH groups are broken away to form free hydrogen sulfide.

Heat-treatment of milk not only produces internal rearrangements of the serum proteins, but also liberates a certain amount of volatile sulfur-containing compounds (Townley and Gould, 31, 32, 33). Such a loss of volatile sulfides must be taken into consideration in studying the effect of heat on the sulfur distribution in milk.

The present research was undertaken to examine the changes produced by heat-treating protein sols in the activity of the —SH groups and in the quantities of sulfur present as volatile sulfur, cysteine, cystine, and methionine. In the main series of experiments, the serum protein sols were heat-treated in nitrogen and the —SH groups determined by titration with o-iodosobenzoate. Supplementary studies were made in which the sols were heated in the presence and absence of air and the —SH groups determined by each of three methods (thiamine disulfide, ferricyanide, and o-iodosobenzoate).

Materials and Methods

The major portion of this investigation deals with a single lot of milk serum protein. Sols of this protein at pH 6.6 were subjected to selected heat-treatments. The volatile sulfides evolved in the process were collected and determined, and the sols were analyzed for titratable sulfhydryl groups. Finally, the sols were dried from the frozen state under vacuum and the dried material was analyzed for cysteine, cystine, methionine, and total sulfur.

Preparation of Milk Serum Proteins. The casein was precipitated from fresh raw, separated milk at 25°C. by the acetate method described in a previous paper (23). The resulting serum was divided

into four portions each of which was dialyzed in Visking sausage casings against running tap water at 13°C. for 42 hours and then against distilled water for 6 hours. A minute amount of protein, precipitated during the dialysis, was removed by filtration. Phosphate buffer was added to each portion to yield a pH of 6.6 and an ionic strength of 0.1 in the final solution. The protein concentrations of these sols were 5 to 6 g. per liter.

Comparisons of the thiamine disulfide, ferricyanide, and o-iodosobenzoate methods for determining sulfhydryl groups were made on another lot of milk serum protein prepared in the same way.

Heat-Treatment and Estimation of Volatile Sulfides. The method employed to heat the serum protein sols was essentially that used by Townley and Gould (31) to heat milk. The apparatus consisted of a 2-liter flask immersed in a water bath and fitted with a condenser, which in turn was connected to a volatile sulfide trap containing 150 ml. of 0.5% zinc acetate in 0.4% sodium hydroxide.

One liter of each of the four sols of serum protein in buffer was successively placed in this apparatus. In each case, the solution, taken from the cold room at 3°C., was brought to 25°C. before it was introduced into the flask. The system was closed and a stream of nitrogen, freed from oxygen and hydrogen sulfide by passing through alkaline pyrogallol and silver nitrate respectively, was bubbled through it. The first lot was maintained at 25°C. for 60 minutes, nitrogen being bubbled through it during 30 minutes and a vacuum drawn with an aspirator during the last 30 minutes. The other three solutions were heated to the respective temperatures of 70°, 75°, or 80°C. in 24 to 28 minutes, maintained at this temperature for 30 minutes, and cooled to 25°C. in 30 minutes. The nitrogen stream was bubbled through the system until the end of the heating period; during the cooling period the nitrogen was stopped and a vacuum was drawn to remove any last traces of volatile sulfides. Sulfides were estimated in the contents of the sulfide collector by the colorimetric method described by Townley and Gould (31). In this method methylene blue is produced from p-aminodimethylaniline, ferric chloride, and the volatile sulfides of the sample. The intensity of the blue color was determined after 1 hour with a Coleman spectrophotometer, employing a wave length setting of 660 mμ.

Titration of Sulfhydryl Groups. After heat-treatment, the milk serum protein sols were dialyzed for 6 hours against tap water and 6 hours against distilled water to remove the buffer. Their protein concentrations changed slightly because of osmosis and consequently were redetermined.

The sulfhydryl groups of these sols were titrated with o-iodoso-

benzoate by the method of Hellerman *et al.* (17, 18). This method depends upon the oxidation at pH 7.0 of the sulfhydryl group to disulfide by o-iodosobenzoate. The excess o-iodosobenzoate is titrated iodimetrically with thiosulfate. It was possible by this method to account for 99% of the cysteine in a 0.100 *N* solution. Titration of egg albumin, prepared by the method of Kekwick and Cannan (22) in 3 *M* guanidine hydrochloride, yielded a value equivalent to 1.30% cysteine, which is in close agreement with the value of 1.29% reported by Hellerman *et al.* (17) for titrations of similar preparations in 7 *M* guanidine hydrochloride.

The titrations of milk serum proteins with the o-iodosobenzoate method were performed as follows: To 10 ml. of the protein solution 5 ml. of phosphate buffer (pH 7.0) and 1 ml. of 0.02 *N* o-iodosobenzoate solution were added. The mixture was shaken for 2 minutes. During the third minute 5 ml. of water, 1.8 ml. of 1 *N* hydrochloric acid, and 2 ml. of starch indicator were added to 0.3 g. of potassium iodide, previously weighed out in a separate flask. At the end of the third minute, the contents of the second flask were added to those of the first and the iodine liberated was titrated with 0.01 *N* sodium thiosulfate using a burette with 0.01 ml. graduations. A blank in which 10 ml. of water was substituted for the 10 ml. of protein solution was also run.

The determinations of sulfhydryl with ferricyanide and thiamine disulfide were made by the methods described by Crowe *et al.* (9) and Harland and Ashworth (14) respectively.

Estimation of Cysteine and Cystine. For the determination of cysteine and cystine 0.3 g. of the dry protein preparations were hydrolyzed for 15 hours at 110°–115°C. with 30 ml. of 6 *N* hydrochloric acid under nitrogen and analyzed by the colorimetric method of Lugg (24, 25) as modified by Kassell and Brand (20) which employs phospho-18-tungstic acid as the oxidant. Some brown humin formed during the hydrolysis. The insoluble portion of this humin was removed by filtration through a sintered glass crucible, and while some soluble humin remained it did not interfere appreciably with the estimation of the blue color of reduced phospho-18-tungstic acid because of the high dilution involved. The absorption of the blue-colored solution was measured with a Coleman spectrophotometer at 670 mμ., the concentration of amino acid being read from a standard curve. In the absence of sulfite the analysis determines cysteine alone. The addition of sulfite causes reduction of cystine to cysteine and consequently both cystine and cysteine are determined if sulfite is added.

In tests on separate solutions of the pure amino acids, recoveries of 95% of the cysteine and 94% of the cystine were realized. When a

solution containing both was analyzed, 92% of the cystine and 99% of the cysteine were recovered. Application of the method to a sample of commercially prepared lactalbumin (S.M.A. Corp., Chagrin Falls, Ohio) yielded 2.80% cystine and 0.21% cysteine, which are in reasonable agreement with the respective values of 2.80% and 0.28% reported by Kassell and Brand (21) for similar material.

Estimation of Methionine. Methionine was determined in the dry proteins by the method of Baernstein (2, 3, 4) which involves hydrolysis of the protein with hydroiodic acid whereupon methyl iodide is formed from the methionine. The gaseous methyl iodide is collected in a bromine solution. Iodate is formed which is titrated with thiosulfate. Samples weighing about 0.5 g. were hydrolyzed with 10 ml. of redistilled hydroiodic acid at 130°–140°C. for 8 hours. Nitrogen was bubbled through the sample during hydrolysis. The evolved gas was washed by passing through a solution of 20% cadmium chloride saturated with respect to mercuric chloride and the methyl iodide was caught in a solution of bromine in acetic acid. At the end of the hydrolysis the excess bromine was reduced with formic acid and an aliquot of the solution titrated iodimetrically with thiosulfate.

A recovery of 93% of pure methionine from solution was obtained with this method.

Determination of Total Sulfur. Total sulfur was determined by oxidizing samples of about 0.25 g. of the dry protein in the Parr peroxide bomb, dissolving the fused mass, and precipitating and weighing the sulfur as barium sulfate.

Results and Discussion

The results of these analyses are given in Table I on an amino acid basis and in Table II on the basis of sulfur distribution.

TABLE I

TITRATABLE SULFHYDRYL GROUPS AND SULFUR-CONTAINING AMINO ACIDS IN MILK SERUM PROTEINS AS AFFECTED BY HEAT-TREATMENT

Heat-treatment[1]	Titratable[2] —SH groups (as cysteine)	Cysteine[3]	Cystine[3]	Methionine[4]
°C.	%	%	%	%
Control	0.87	2.9	0.0	3.2
70	0.68	—	—	—
75	0.51	—	—	—
80	0.42	3.0	0.0	3.1

[1] The heat-treated samples were raised to the indicated temperatures in 24 to 28 minutes, held at the specified temperature for 30 minutes, and cooled to 25°C. in 30 minutes.
[2] Mean of three determinations by the o-iodosobenzoate method.
[3] Mean of six determinations, three on one weighed sample and three on another.
[4] Mean of three determinations on the hydrolyzate from one weighed sample.

TABLE II

SULFUR DISTRIBUTION OF UNDENATURED AND HEAT-TREATED MILK SERUM PROTEINS

Heat-treatment[1]	Volatile sulfur[2]	Cysteine sulfur[3]	Methionine sulfur[4]	Total sulfur[5]
°C.	%	%	%	%
Control	0.000013	0.78	0.69	1.6
70	0.000022	—	—	1.6
75	0.000031	—	—	1.6
80	0.000032	0.79	0.67	1.6

[1] The heat-treated samples were raised to the indicated temperatures in 24 to 28 minutes, held at the specified temperature for 30 minutes, and cooled to 25°C. in 30 minutes.
[2] Single determination.
[3] Mean of six determinations, three on one weighed sample and three on another.
[4] Mean of three determinations or the hydrolyzate from one weighed sample.
[5] Mean of duplicate determinations on separately weighed samples.

The titratable sulfhydryl groups decreased progressively as the heat-treatment was made more drastic. However, no change occurred in the cysteine content as determined in the hydrolyzate. In fact, all of the nonmethionine sulfur of the protein appeared to be in the form of cysteine regardless of heat-treatment. This result was very surprising in view of the reports of Kassell and Brand (21) and Brand *et al.* (7) that the major portion of the nonmethionine sulfur is present as cystine in "lactalbumin" and β-lactoglobulin respectively. The possibility that this apparent anomaly was due to reduction occurring during the hydrolysis or during the subsequent determinations was precluded by a test in which 22.5 mg. of cystine was added to the protein sample before hydrolysis and 94.5% of this amount was recovered by the analysis.

The data obtained for cysteine agreed satisfactorily with those obtained by others for the sum of the cysteine and cystine in milk serum proteins (5, 6). The methionine percentage is in reasonable agreement with other data (5, 6) and the sum of the cysteine and methionine sulfur represents about 91% of the total sulfur.

The amount of sulfur volatilized is of the same order of magnitude as that found by Townley and Gould (31) upon heating milk. For example, they found that an amount of sulfur equivalent[4] to 0.000015% of the serum protein was volatilized during heating milk at 80°C. for 30 minutes. The results in Table II show that 0.000032% sulfur was volatilized by subjecting serum protein to the same treatment. The fact that a lower value is obtained by heating milk is in accord with the finding of Townley and Gould (33) that the presence of sugar reduces the volatilization of sulfides during heating.

The iodosobenzoate titration yields higher values for the sulfhydryl

[4] Calculated on the assumption that one liter of milk contains 7.3 g. of serum protein.

content of unheated milk serum protein than has been obtained by the use of either thiamine disulfide or of ferricyanide as oxidants. Thus, Harland *et al.* (16) found that thiamine disulfide oxidized only a negligible number of groups in unheated milk serum protein. Crowe *et al.* (9) and Harland *et al.* (16) have reported values obtained on milk serum protein by the ferricyanide method which are equivalent to 0.05% and 0.29% [5] cysteine respectively. A direct comparison of the three methods on a single sample of milk serum proteins is afforded by the data of Table III. These data were secured on a sample of protein prepared in the manner previously indicated. Portions were heat-treated in stainless steel tubes at 75°C. and 85°C. for 30 minutes in an atmosphere either of air or of nitrogen[6] and the sulfhydryl determina-

TABLE III

EFFECT OF HEAT-TREATMENT ON THE SULFHYDRYL GROUPS IN MILK SERUM PROTEIN AS DETERMINED BY VARIOUS METHODS [1]

Heat-treatment for 30 min.	—SH groups (as cysteine) by various methods		
	Thiamine disulfide	Ferricyanide	o-iodosobenzoate
°C.	%	%	%
Control	0.02	0.09	0.63
HEATED IN AIR			
75	0.08	0.18	0.60
85	0.12	0.21	0.32
HEATED IN NITROGEN			
75	0.10	0.25	0.67
85	0.20	0.34	0.68

[1] Sol of protein was buffered at pH 6.6 with phosphate buffer having an ionic strength of 0.1. The protein concentration of the sol was 5.9 g. per liter.

tions were made immediately after heating and cooling. Comparison of results obtained by the three methods on the unheated samples suggests that the sulfhydryl groups of the protein are of graded reactivity or availability. Thus the number of groups oxidized depends on the oxidation-reduction potential of the oxidant and on the conditions employed.

Heat-treatment of the serum protein caused a decrease in the number of groups titratable by iodosobenzoate. On the other hand,

[5] These values were computed from the data graphed in Fig. 6 of the paper by Crowe *et al.* (9) and in Fig. 3 of that of Harland *et al.* (16). The sample used by the latter was prepared from nonfat dry milk solids spray-dried from milk that had been preheated at 63°C. for 30 minutes; this treatment undoubtedly explains its higher capacity to reduce ferricyanide. Two other samples of serum protein isolated from fresh skimmilk and analyzed by this method in the laboratories of the Division of Dairy Husbandry yielded values equivalent to 0.09 and 0.10% cysteine respectively.

[6] This sample was prepared and heat-treated by H. A. Harland. The thiamine disulfide and ferricyanide determinations were also performed by him

it has been shown that heat-treatment results in increases in the groups available to thiamine disulfide and ferricyanide (9, 16). The appearance of groups which reduce thiamine disulfide coincides with the appearance of a positive nitroprusside test. Evidently a small number of groups are made more active than were any groups originally.[7]

Obviously, the decrease which heat-treatment produces in the power to reduce iodosobenzoate cannot be due to loss of the insignificant amount of sulfur volatilized during heating. Another possibility is that the decrease in titratable sulfhydryl groups represents oxidation during or following heating. This possibility gains support from the facts that the groups which reduce thiamine disulfide are known to be susceptible to oxidation, and that in the present study, the protein sols for which data are reported in Tables I and II were protected from oxygen during the heat-treatment but were exposed to air in the subsequent dialysis and storage prior to analysis. It gains even more support from the data of Table III which show definitely that when the titration is performed immediately after heating and cooling the decrease in groups available to o-iodosobenzoate does not occur in the absence of oxygen during heat-treatment. However, the failure to detect cystine in hydrolyzates of either unheated or heated samples seems to preclude the possibility of explaining the decrease in titer on the basis of oxidation of sulfhydryl to disulfide linkages. This whole question of the possible role of oxidation is receiving further study at present.

In addition to oxidation, heat-treatment undoubtedly causes an unfolding of the protein molecules such that, upon cooling, the molecule refolds or agglomerates with other molecules in such a way that groups originally available to oxidation by iodosobenzoate are made sterically unavailable. This affords an alternate or additional explanation for the decrease in titration.

It is not known, of course, to what extent the changes produced by heat are due respectively to interactions among different species of protein micelles, to interactions among micelles of a given species, and to strictly intramolecular rearrangements. Since milk serum protein is a mixture of several electrophoretically different components (10, 29), the possibility of interaction is evident. Unpublished observations by one of us (R. J.) indicate that heat-treatment of milk serum protein in phosphate buffer at pH 6.9 and an ionic strength of 0.1 at 100°C. for 30 minutes causes the mixture to become electrophoretically more homogeneous, thus supporting the hypothesis that interaction can and does occur. On the other hand, Briggs and Hull (8) reported

[7] The maximum observed by Harland *et al.* (16) was attained on heating at 95°C. for 2 minutes. It was equivalent to 0.33% cysteine.

that continuous aggregation occurs during heat-treatment of purified β-lactoglobulin. A few titrations made by the iodosobenzoate method on some of the β-lactoglobulin samples prepared by Briggs and Hull indicated that a decrease in titratable sulfhydryl groups is produced by heat-treatment of this rather homogeneous protein.

There is no assurance that the changes that have been found to occur in sols of milk serum protein in phosphate buffer are duplicated when these proteins are heated in their natural environment in milk. If oxidation is indeed a significant factor in the heat-induced changes, then the alterations produced in milk heated in the presence of air may be different from those recorded in this paper where air was excluded, at least during the heating process. In milk, moreover, the protein sulfhydryl groups may react with other nonprotein constituents. It has been demonstrated that when lactose is present in the system, heating produces an interaction between the proteins and lactose which results in a larger increase in capacity to reduce ferricyanide than that resulting from heating the protein alone (9, 16). Interestingly enough, lactose either retards the liberation by heat of groups that reduce thiamine disulfide or reacts with some of such liberated groups (16).

The decrease which heating produces in sulfhydryl groups titratable with iodosobenzoate coincides with the loss of dough-softening action which was shown in a previous paper to occur upon heating of sols of milk serum protein. Thus, these data agree with the suggestion of Stamberg and Bailey (30) that heat-treatment reduces the over-all activity of the sulfhydryl groups of milk serum protein and that this in turn causes the loss of dough-softening action and the improvement in baking quality of nonfat dry milk solids.

Acknowledgments

The authors are grateful to the American Dry Milk Institute, Chicago, Illinois, for a grant in support of this work. They are also indebted to H. A. Harland of the Division of Dairy Husbandry, University of Minnesota, for making some of the analyses.

Literature Cited

1. Anson, M. L. Protein denaturation and the properties of protein groups. Advances in Protein Chem. **2**: 361–386 (1945).
2. Baernstein, H. D. The determination of methionine in proteins. J. Biol. Chem. **97**: 663–668 (1932).
3. Baernstein, H. D. The sulfur distribution in proteins. J. Biol. Chem. **97**: 669–674 (1932).
4. Baernstein, H. D. A new method for the determination of methionine in proteins. J. Biol. Chem. **115**: 25–32 (1936).
5. Beach, E. F., Bernstein, S. S., Hoffman, O. D., Teague, D. M., and Macy, I. G. Distribution of nitrogen and protein amino acids in human and in cow's milk. J. Biol. Chem. **139**: 57–63 (1941).
6. Block, R. J., and Bolling, Diana. The amino acid composition of cow and human milk proteins. Arch. Biochem. **10**: 359–363 (1946).
7. Brand, E., Saidel, L. J., Goldwater, W. H., Kassell, Beatrice, and Ryan,

F. J. The empirical formula of β-lactoglobulin. J. Am. Chem. Soc. **67**: 1524–1532 (1945).
8. Briggs, D. R., and Hull, R. Studies on protein denaturation. I. Electrophoretic study kinetics at neutrality of heat denaturation of β-lactoglobulin. J. Am. Chem. Soc. **67**: 2007–2014 (1945).
9. Crowe, L. K., Jenness, R., and Coulter, S. T. The reducing capacity of milk and milk products as measured by a modified ferricyanide method. J. Dairy Sci. **31**: 595–610 (1948).
10. Deutsch, H. F. A study of whey proteins from the milk of various animals. J. Biol. Chem. **169**: 437–448 (1947).
11. Greenbank, G. R., Steinbarger, Mabel C., Deysher, E. F., and Holm, G. E. The effect of heat treatment of skim milk upon the baking quality of the evaporated and dried products. J. Dairy Sci. **10**: 335–342 (1927).
12. Grewe, Emily, and Holm, G. E. Effect of variation in the method of manufacture on the baking quality of dry skimmilk. Cereal Chem. **5**: 461–469 (1928).
13. Gould, I. A., and Sommer, H. H. Effect of heat on milk with especial reference to the cooked flavor. Mich. Agr. Expt. Sta. Tech. Bull. 164 (1939).
14. Harland, H. A., and Ashworth, U. S. The use of thiamin disulfide for the estimation of reducing substances in processed milk. J. Dairy Sci. **28**: 15–23 (1945).
15. Harland, H. A., Ashworth, U. S., and Golding, N. S. Chemical factors affecting the baking quality of dry milk solids. III. The effect of several milk fractions on loaf volume. Cereal Chem. **20**: 535–542 (1943).
16. Harland, H. A., Coulter, S. T., and Jenness, R. Some factors influencing the reducing systems in dry whole milk. J. Dairy Sci. **32**: 334–344 (1949).
17. Hellerman, L., Chinard, F. P., and Ramsdell, Pauline A. O-iodosobenzoic acid, a reagent for the estimation of cysteine, glutathione, and the substituent sulfhydryl groups of certain proteins. J. Am. Chem. Soc. **63**: 2551–2553 (1941).
18. Hellerman, L., Chinard, F. P., and Deitz, V. R. Protein sulfhydryl groups and the reversible inactivation of the enzyme urease. The reducing groups of egg albumin and of urease. J. Biol. Chem. **147**: 443–462 (1943).
19. Josephson, D. V., and Doan, F. J. Observations on cooked flavor in milk, its source and significance. Milk Dealer **29**(2): 35–36, 54–62 (1939).
20. Kassell, Beatrice, and Brand, E. The photometric determination of cystine, cysteine, ascorbic acid, and related compounds with phosphotungstic acid. J. Biol. Chem. **125**: 115–129 (1938).
21. Kassell, Beatrice, and Brand, E. The distribution of the sulfur in casein, lactalbumin, edestin, and papain. J. Biol. Chem. **125**: 435–443 (1938).
22. Kekwick, R. A., and Cannan, R. K. The hydrogen ion dissociation curve of the crystalline albumin of the hen's egg. Biochem. J. **30**: 227–234 (1936).
23. Larsen, R. A., Jenness, R., and Geddes, W. F. Effect of heat treatment of separated milk on the physical and baking properties of doughs enriched with dry milk solids. Cereal Chem. **26**: 189–200 (1949).
24. Lugg, J. W. H. The application of phospho-18-tungstic acid (Folin's reagent) to the colorimetric determination of cysteine, cystine and related substances. I. The reduction of phospho-18-tungstic acid by various substances. Biochem. J. **26**: 2144–2159 (1932).
25. Lugg, J. W. H. The application of phospho-18-tungstic acid (Folin's reagent) to the colorimetric determination of cysteine, cystine and related substances. II. The determination of sulphydryl compounds and disulfides already existing in solution. Biochem. J. **26**: 2160–2165 (1932).
26. Neurath, H., Greenstein, J. P., Putnam, F. W., and Erickson, J. O. The chemistry of protein denaturation. Chem. Revs. **34**: 157–265 (1944).
27. Shen, T., and Geddes, W. F. Protease activity and reducing matter content of wheat flour doughs in relation to baking behavior. Cereal Chem. **19**: 609–631 (1942).
28. Skovholt, O., and Bailey, C. H. The effect of temperature and of the inclusion of dry skimmilk upon the properties of doughs as measured with the farinograph. Cereal Chem. **9**: 523–530 (1932).
29. Smith, E. L. Isolation and properties of immune lactoglobulins from bovine whey. J. Biol. Chem. **165**: 665–676 (1946).
30. Stamberg, O. E., and Bailey, C. H. The effect of heat treatment of milk in

relation to baking quality as shown by polarograph and farinograph studies. Cereal Chem. 19: 507–517 (1942).
31. TOWNLEY, R. C., and GOULD, I. A. A quantitative study of heat labile sulfides of milk. I. Method of determination and the influence of temperature and time. J. Dairy Sci. 26: 689–703 (1943).
32. TOWNLEY, R. C., and GOULD, I. A. A quantitative study of heat labile sulfides of milk. II. General origin of sulfides and relation to total sulfur, total nitrogen and albumin nitrogen. J. Dairy Sci. 26: 843–851 (1943).
33. TOWNLEY, R. C., and GOULD, I. A. A quantitative study of heat labile sulfides of milk. III. Influence of pH, added compounds, homogenization and sunlight. J. Dairy Sci. 26: 853–867 (1943).

BIOLOGICAL VALUE OF THE PROTEIN AND THE MINERAL, VITAMIN, AND AMINO ACID CONTENT OF SOYMILK AND CURD [1]

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ABSTRACT

Soymilk was prepared by soaking the beans in water overnight followed by draining and grinding. Water was then added, the whole heated to 200°F., and solid matter filtered off. The curd was precipitated from the milk by the addition of magnesium chloride. Soymilk powder was prepared from the milk in a spray drier. The composition was then determined. The biological value of the protein of these products was assayed by the rat growth and nitrogen retention methods. The soymilk was 80% as efficient as whole milk powder, whereas the comparative value for the whole bean and curd was 75%. Supplementing soybean protein with sesame seed raised the value to 94%. The soymilk and curd contained approximately 0.25 to 0.34% of calcium, 0.01 to 0.015% of iron, and 0.8 to 1.0% of phosphorus. The curd had a higher percentage of these minerals than the original bean. Soymilk contained 11.8 μg. thiamine, 4.6 μg. riboflavin, and 29.0 μg. nicotinic acid per g.; the curd 3.9 μg. thiamine, 3.7 μg. riboflavin, and 5.5 μg. nicotinic acid per g. These figures were calculated on a moisture-free basis. The milk was a better source of these vitamins than the original bean. Soymilk contained 1.4% methionine and 2.4% of cystine; the corresponding values for the curd were 1.2 and 1.2% respectively.

Soybeans have long been regarded as a nutritious food. They are not only unusually high in protein (40%) and oil (20%), but are also good sources of the minerals—iron, calcium, and phosphorus—and the B vitamins (Payne and Stuart, 29).

Numerous investigations have been conducted on the biological value of soybean proteins. Work on men and dogs (25), rats (5, 18, 27, 28), and chicks (1, 2, 14) has indicated that proteins of adequately heated soybeans are high in value.

[1] Manuscript received October 28, 1948.
Published as Scientific Paper No. 184, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington. Submitted in partial fulfillment of the requirements for the Ph.D. degree in Home Economics.
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Sprague (34) reported that soybeans contain substantial quantities of thiamine (0.8 to 1.3 mg. per 100 g.), niacin (4.8 to 9.0 mg. per 100 g.), and riboflavin (0.3 to 0.4 mg. per 100 g.). The results obtained by Burkholder (7) were in good agreement with these findings.

Bailey, Capen, and LeClerc (4) gave the following percentages for the mineral content of soybeans on an air-dried basis: potassium 1.9, sodium 0.3, calcium 0.2, magnesium 0.2, phosphorus 0.6, sulfur 0.4, and chlorine 0.02. Sherman, Elvehjem, and Hart (32) found that of the 0.01% of iron present in roasted soybeans 80% was available to the animal organism.

In this country soybeans are used mostly for animal feeds, and only small amounts are consumed as human food. Because of the high nutritive value, attempts are being made to adapt them to the American diet.

In the Orient soybeans have served as the sustaining food for centuries and have long been favored as "poor man's meat." In these countries they are consumed chiefly in the form of soymilk and soybean curd. Soymilk is a water extraction from soybeans, similar in composition and appearance to cow's milk, and the curd, produced by precipitating the protein from soymilk, has a texture somewhat like cream cheese.

Despite the fact that considerable work has been done on the nutritive value of soybeans, relatively little has been reported on soymilk and curd. The purpose of this paper was twofold: (1) to compare the biological value of the protein of the soymilk and curd with that of the original bean and whole milk powder and (2) to determine the vitamin, mineral, and amino acid content of these products.

Part I. Biological Value of Soybean Products

Cahill *et al.* (8) worked on human adults and found that soymilk was utilized for maintenance as well as cooked soybeans. Cheng (9) using the nitrogen-balance method with puppies observed that the biological value of soybean curd was only 70% as efficient as that of the raw bean. His results indicated that the value of the protein in the raw and cooked bean was about the same; a finding contrary to that of many workers. Desikachar (12) compared the biological value of soymilk protein with cow's milk and found that the former was about 90% as efficient as the latter.

In the present work rats were used to compare the protein efficiency of soymilk and curd with cooked bean and whole milk powder by the growth and nitrogen retention method.

Materials and Methods

Preparation of Soymilk and Soybean Curd. Mature, air-dry, Funk Delicious soybeans were used for all experiments. The milk was prepared according to the traditional methods used in China. The beans were soaked with four volumes of water overnight at room temperature (22°C.); they absorbed about one and one-half volumes of water. They were drained and ground. A quantity of water equivalent to four and one-half times the weight of the original dry bean was added and the mixture heated to 95°C. Milk was then obtained by filtering through a double thickness of cheese cloth.

The method of preparation used in France as described by Chin (10) was also tried, but the results were not as satisfactory. Since the bean was ground without previous soaking in this case, it was brittle and broke into particles of various sizes, some of which passed through the cheese cloth during filtration and a suspension instead of an emulsion resulted. Grinding the soaked bean produced flakes rather than powdery particles. Proximate analyses were carried out on the soybeans and the products (Table I).

TABLE I

COMPOSITION OF SOYBEANS, MILK, AND CURD

Constituents	Soymilk		Soybean curd		Soybean	
	Wet basis	Moisture-free basis	Wet basis	Moisture-free basis	Wet basis	Moisture-free basis
	%	%	%	%	%	%
Moisture	91.0	—	76.5	—	5.3	—
Protein[1]	4.4	48.4	15.0	63.7	41.4	43.7
Fat (ether extract)	1.7	19.0	7.1	30.0	21.0	22.2
Ash	0.5	5.7	0.9	3.6	4.6	4.9
Carbohydrate (by difference)	2.4	26.9	0.6	2.7	27.6	29.2

[1] Calculated using a factor of 6.25.

Animal Assay. Soymilk powder, soy curd, and soybeans were studied. The soymilk powder was prepared by drying the milk in an air spray dryer to powder form. The curd was frozen and dehydrated at 60°C. Since maximal biological value of soybean protein is obtained by autoclaving at 110°C. for 30 minutes (Evans and McGinnis, 14), beans were treated at this temperature for this period of time. The autoclaved curd was prepared in a similar manner. All dried products were ground, then analyzed for moisture and nitrogen using the Kjeldahl-Gunning-Arnold method (3). The quantity of each required to furnish a protein level (N × 6.25) of 10% was used in

making the diets (Table II). Due to a high methionine content, sesame seed was added as a supplement to the autoclaved bean and curd diets. A diet containing whole milk powder at a 10% protein level was the standard for comparison.

TABLE II

COMPOSITION OF DIETS

Ingredients	Whole milk powder	Soymilk powder	Autoclaved soybean	Soybean curd	Autoclaved curd	Autoclaved soybean plus sesame meal	Curd plus sesame meal
	%	%	%	%	%	%	%
Whole milk powder	37.5	—	—	—	—	—	—
Soymilk powder	—	23.2	—	—	—	—	—
Autoclaved soybean	—	—	26.6	—	—	17.7	—
Dried soybean curd	—	—	—	17.9	—	—	12.0
Autoclaved curd	—	—	—	—	17.9	—	—
Sesame meal	—	—	—	—	—	7.1	7.1
Starch	60.0	68.6	66.5	74.8	74.9	68.9	74.4
Hydrogenated fat	—	5.7	4.4	4.8	4.7	3.8	4.0
Hubbell salts[1]	2.5	2.5	2.5	2.5	2.5	2.5	2.5

[1] Hubbell *et al.* (20).

Three times a week each rat received as a supplement 1 ml. of a vitamin solution containing thiamine hydrochloride 50 μg., nicotinic acid 580 μg., inositol 130 μg., choline hydrochloride 20 mg., riboflavin 50 μg., pyridoxine 50 μg., calcium pantothenate 50 μg., p-aminobenzoic acid 250 μg., and ⅓ ml. of a fat-soluble vitamin solution containing vitamin A 1,000 I.U. and D 100 I.U., and mixed tocopherols 2 mg. per ml.

Young albino rats, 28 to 31 days of age, were selected and paired as to sex and size into seven groups. They were housed in individual cages, weighed three times weekly, and fed *ad libitum*. Trials were run for 5 weeks.

At the conclusion of the experiment animals were killed, the gastrointestinal tract removed, and the carcass digested in 9% hydrochloric acid over a steam bath for 48 hours. The digest was homogenized in a Waring Blendor, made to volume and nitrogen determined on aliquots. Nitrogen storage was obtained by subtracting the nitrogen content of a comparable group analyzed at the beginning of the experiment from that of the experimental group.

Results

Growth studies are summarized in Table III and protein retention in Table IV; a statistical comparison of the figures is shown in Table V.

Animals on the soymilk diet ate more than those receiving whole milk powder and as a consequence a significantly higher gain resulted.

TABLE III

GROWTH RESPONSE ON DIETS USED TO DETERMINE BIOLOGICAL VALUE OF SOYBEAN PRODUCTS

Diets	Number of animals	Average food eaten daily per rat	Average total gain	Average gain per gram of protein	Average food required for gram of gain	Per cent[1] efficiency
		g.	g.	g.	g.	
Whole milk powder	8	10.5	77.1±3.6[2]	2.30±0.06[2]	4.80±0.12[2]	100
Soymilk powder	8	12.4	95.1±6.9	1.88±0.07	4.64±0.17	82
Autoclaved soybean	8	11.8	80.9±3.2	1.73±0.05	5.13±0.13	75
Soybean curd	8	10.7	71.0±4.4	1.73±0.05	5.31±0.16	75
Autoclaved soybean curd	8	9.9	61.8±5.1	1.59±0.09	5.77±6.34	69
Autoclaved soybean + sesame seed	8	12.0	99.8±7.7	2.17±0.09	4.34±0.23	94
Soybean curd + sesame seed	8	11.3	91.0±5.8	2.10±0.10	4.45±0.25	91

[1] Calculated from protein efficiency of each diet using whole milk powder as 100.
[2] Standard error.

However, the protein efficiency of the whole milk powder was higher than that of soymilk, bean, or curd; statistical analysis gave a "t" value of 4.6 for milk versus soymilk, 7.7 for milk versus soybean, and 7.5 for milk versus curd; these differences are highly significant. The soymilk appeared to be slightly better in quality than the whole bean. Growth on the soymilk diet was significantly better than on the curd, but this was not true of the efficiency. The protein efficiency of the whole bean and curd was the same. Autoclaving did not improve the curd. Jones and Widness (21) report a protein efficiency of 2.64 for skim milk powder and Block and Mitchell (6) cite a figure of 2.8, values higher than the one secured in this study with whole milk powder.

TABLE IV

NITROGEN RETENTION ON DIETS USED TO DETERMINE BIOLOGICAL VALUE OF SOYBEAN PRODUCTS

Diets	Number of animals	Average nitrogen retention	Average retention per gram of nitrogen	Per cent[1] efficiency
		g.	g.	
Whole milk powder	8	1.97±0.13[2]	0.37±0.01[2]	100
Soymilk powder	8	2.37±0.19	0.29±0.02	79
Autoclaved soybean	8	1.99±0.01	0.27±0.01	73
Soybean curd	8	1.80±0.10	0.27±0.01	73
Autoclaved soybean curd	8	1.48±0.11	0.24±0.01	65
Autoclaved soybean + sesame seed	8	2.60±0.18	0.36±0.02	97
Soybean curd + sesame seed	8	2.44±0.12	0.35±0.01	95

[1] Calculated using whole milk powder as 100.
[2] Standard error.

TABLE V

STATISTICAL COMPARISONS OF GROWTH AND NITROGEN RETENTION ON VARIOUS DIETS

Diets compared	Weight gain (g.)		Gram gain per gram protein		Total nitrogen retention (g.)		Nitrogen retention per gram nitrogen	
	Mean difference	t[1]	Mean difference	t[1]	Mean difference	t[1]	Mean difference	t[1]
Milk vs. soymilk	18.0	2.32*	0.42	4.61**	0.40	1.84	0.08	3.95**
Milk vs. autoclaved soybean	3.8	0.79	0.57	7.70**	0.02	0.86	0.10	5.65**
Milk vs. curd	6.1	1.07	0.57	7.50**	0.18	0.96	0.10	6.76**
Soymilk vs. autoclaved soybean	14.2	1.88	0.15	1.76	0.39	1.80	0.02	1.05
Soymilk vs. curd	24.1	2.96**	0.15	1.70	0.58	2.71*	0.02	1.25
Autoclaved soybean vs. curd	9.9	1.83	0.00	0.00	0.19	1.07	0.00	0.00
Curd vs. autoclaved curd	9.2	1.37	0.14	1.31	0.31	1.69	0.03	2.66*
Autoclaved soybean + sesame seed vs. autoclaved bean	18.9	2.27*	0.44	4.26**	0.62	2.89*	0.09	4.68**
Curd + sesame vs. curd	20.0	2.74*	0.37	3.59**	0.64	3.43**	0.08	5.12**
Milk vs. autoclaved soybean + sesame	22.7	2.67*	0.13	1.20	0.63	2.94*	0.01	0.49
Milk vs. curd + sesame	13.9	2.04	0.20	1.64	0.47	3.83**	0.01	1.19

[1] Calculated from Fisher's formula (Snedecor, 33).

The addition of sesame seed to both autoclaved soybean and curd increased growth but not protein efficiency above that obtained on milk powder.

In general, the correlation between values resulting from growth, protein efficiency, and nitrogen retention was good with the exception that soymilk produced significantly greater growth than milk powder, but both the efficiency and nitrogen retention were significantly lower.

Part II. Vitamin Mineral and Amino Acid Content of Soybean Products

With the exception of the work of Miller (26) on the thiamine content of soybean curd, few references are found in the literature on this subject. According to his report only 20% of the thiamine in the bean was retained in the curd. Since soybeans are considered rich sources of B vitamins—thiamine, riboflavin, and nicotinic acid—retention in the milk and curd was investigated.

No information on the mineral content of soybean products was found. Because the content of phosphorus, calcium, and iron in soybeans is high, investigation of the retention of these nutrients in soymilk and curd was considered desirable.

Soybean and the products were analyzed for methionine since various workers have demonstrated that the amount of this amino acid limits the biological value of soybean protein.

Materials and Methods

Thiamine was determined by the method of Conner and Straub (11) modified to include extraction of the samples with isobutyl alcohol to remove nonthiochrome fluorescing materials (Harris and Wang, 17). The method of Peterson, Brady, and Shaw (30) was used for riboflavin, modified by the use of 0.1 *N* sulfuric acid for extraction of the samples. Nicotinic acid was determined by microbiological assay employing a dehydrated medium prepared by a commercial laboratory.[4] Sodium hydroxide was used for extraction of the sample as described by Teply, Strong, and Elvehjem (35).

Minerals were determined on the dry sample by wet ashing with perchloric acid according to the method of Gerritz (16). Calcium was determined by ceric sulfate titration using the method of Kirk and Schmidt (23) with the modification of Kirk and Moberg (22). Fiske and Subbarow's colorimetric method (15) was employed for the determination of phosphorus. Iron was analyzed by the colorimetric method of Koenig and Johnson (24).

Methionine was assayed by two methods: the differential oxidation method of Evans (13), and the microbiological method of Henderson and Snell (19). Using the Evans procedure, some methionine apparently is oxidized along with the cystine during nitric acid digestion, resulting in low recovery for the former (90%) and high for the latter (110%); accordingly this digestion period was cut from 24 to 16 hours.

For microbiological assay, samples were prepared by hydrolyzing with 2 *N* hydrochloric acid in the autoclave at 15 lb. pressure for 5 hours. Longer treatment did not release more of the amino acids but resulted in some destruction. Henderson and Snell's medium was employed with the following modifications: 100 times as much folic acid was added to the medium in order to increase the acid production to a maximum at high levels of methionine when *Leuconostoc mesenteroides* was used as the assay organism and DL-glutamic acid was used instead of the L-isomer because the latter was found to contain traces of methionine which interfered with the determination. When these changes were employed, smooth standard curves with low blanks and high acid production were obtained repeatedly with *Leuconostoc mesenteroides* P-60[4] and *Lactobacillus arabinosus* 17-5[4].

Results

The results of vitamin, mineral, and amino acid analyses in the soybean and its products are shown in Tables VI, VII, and VIII. The retention of minerals and vitamins in these samples was compared on a moisture- and fat-free basis.

[4] Difco Laboratories, Inc., Detroit, Michigan.

TABLE VI

THIAMINE, RIBOFLAVIN, AND NICOTINIC ACID CONTENT OF SOYBEAN, MILK, AND CURD[1]

Products	Moisture-free basis μg./g.			Moisture- and fat-free basis μg./g.		
	Thiamine	Riboflavin	Nicotinic acid	Thiamine	Riboflavin	Nicotinic acid
Soybean (raw)	8.7	3.2	15.9	11.3	4.1	21.0
Soymilk	11.8	4.6	29.0	14.8	6.1	35.0
Soybean curd	3.9	3.7	5.5	6.6	5.0	6.0

[1] Average of duplicate determinations.

TABLE VII

CALCIUM, IRON, AND PHOSPHORUS CONTENT OF SOYBEAN, MILK, AND CURD

Products	Moisture-free basis %			Moisture- and fat-free basis %		
	Calcium	Iron	Phosphorus	Calcium	Iron	Phosphorus
Soybean (raw)	0.205	0.0078	0.57	0.263	0.0100	0.73
Soymilk[1]	0.195	0.0072	0.65	0.241	0.0089	0.80
Soybean curd[1] ($MgCl_2$ precipitated)	0.241	0.0105	0.80	0.344	0.0150	1.07
Soybean curd ($CaCl_2$ precipitated)	0.690	—	—	0.990	—	—

[1] Analyses on soymilk and curd were run on pooled samples from several preparations.

TABLE VIII

METHIONINE AND CYSTINE CONTENT OF PROTEIN OF SOYBEAN, MILK, AND CURD

Products	Differential oxidation method[1]		Microbiological method[1]	
			Methionine[2]	
	Methionine[2]	Cystine[2]	Organism employed	
			Leuconostoc mesenteroides	*Lactobacillus arabinosus*
	%	%	%	%
Soybean (raw)	1.14	1.83	1.14	0.78
Soymilk	1.36	2.32	1.16	0.85
Curd	1.22	1.19	1.05	0.77
Sesame seed	3.35	2.74	—	—

[1] Calculated to 16% nitrogen.
[2] Average value of duplicate determinations.

Higher concentrations of the three vitamins were found in the soymilk. This could be anticipated due to their solubility in water. Only about one-half of the thiamine and one-fourth of the nicotinic

acid were retained in soybean curd. Probably these were lost by solution when the curd was separated from the whey. The riboflavin content was equivalent to that of the original bean.

The mineral content in soymilk was comparable to that of the bean, whereas higher concentrations of calcium, iron, and phosphorus were found in the curd. Since the dairy industry is not well developed in China, the adequacy of calcium in the diet has been considered one problem in the nutrition of the Chinese. In order to introduce more calcium, an effort was made to enrich the curd with this mineral. The soymilk was precipitated with a solution of calcium sulfate or chloride instead of magnesium salt. The resulting products apparently were not different in texture and taste, while the calcium content was raised from 0.24 to 0.69 g. per 100 g. Schroeder, Cahill, and Smith (31) reported that calcium from soymilk is 80% as available for human utilization as the calcium in cow's milk. The use of soybean curd precipitated by a calcium salt should be helpful in increasing the amount of this mineral in the Chinese diet.

The methionine content of soybean products as determined by the differential oxidation method agreed fairly well with the microbiological assay using *Leuconostoc mesenteroides*, but was higher than results obtained by using *Lactobacillus arabinosus*. High concentrations of sodium chloride resulting from the neutralization of hydrochloric acid used for digestion were not responsible for this difference. When casein was assayed for methionine using *Lactobacillus arabinosus*, low results were again obtained. From these findings the conclusion may be drawn that the use of *Leuconostoc mesenteroides* is preferable for methionine assays.

Literature Cited

1. Almquist, H. J. Proteins and amino acids in animal nutrition. F. E. Booth Company, Inc.: New York (1946).
2. Almquist, H. J., Mecchi, E., Kratzer, F. H., and Grau, C. R. Soybean protein as a source of amino acids for the chick. J. Nutrition **24**: 385–392 (1942).
3. Association of Official Agricultural Chemists. Official and tentative methods of analysis (6th ed.). Assoc. Official Agr. Chemists: Washington, D. C. (1945).
4. Bailey, L. H., Capen, R. G., and LeClerc, J. A. The composition and characteristics of soybeans, soybean flour, and soybean bread. Cereal Chem. **12**: 441–472 (1935).
5. Baldwin, F. M., and Movitt, E. J. Nutritional value of autoclaved and raw soybeans, supplanted only by vitamins and salt mixture. Food Research **7**: 403–404 (1942).
6. Block, R. J., and Mitchell, H. H. The correlation of the amino-acid composition of proteins with their nutritive value. Nutrition Abstracts & Revs. **16**: 249–278 (1946–47).
7. Burkholder, P. R. Vitamins in edible soybeans. Science **98**: 188–190 (1943).
8. Cahill, W. M., Schroeder, L. J., and Smith, A. H. Digestibility and biological value of soybean protein in whole soybeans, soybean flour and soybean milk. J. Nutrition **28**: 209–218 (1944).

9. CHENG, W. K. The digestibility and biological value of raw soybean, heated soybean, and the soybean-milk clot. Biochem. Bull. (China) **41**: 1–3 (1944).
10. CHIN, K. C. Nature of the vegetable milk from soybeans and the precautions taken in its use. Chem. Abstracts **41**: 3880–3881. Compt. rend. **224**: 288–290 (1947).
11. CONNER, R. T., and STRAUB, G. J. Determination of thiamin by the thiochrome reaction. Ind. Eng. Chem. (Anal. Ed.) **13**: 380–384 (1941).
12. DESIKACHAR, H. S. R., DE, S. S., and SUBRAHMANYAN, V. The nutritive value of soybean milk. I. Nutritive value of the protein. Ann. Biochem. Exptl. Med. (India) **6**: 49–56 (1946).
13. EVANS, R. J. Determination of the cystine and methionine content of plant and animal material by a differential oxidation procedure. Arch. Biochem. **7**: 439–445 (1945).
14. EVANS, R. J., MCGINNIS, J., and ST. JOHN, J. L. The influence of autoclaving soybean oil meal on the digestibility of the proteins. J. Nutrition **33**: 661–672 (1947).
15. FISKE, C. H., and SUBBAROW, Y. The colorimetric determination of phosphorus. J. Biol. Chem. **66**: 375–400 (1925).
16. GERRITZ, H. W. Digesting biological materials for calcium and phosphorus analysis. Ind. Eng. Chem. (Anal. Ed.) **7**: 167–168 (1935).
17. HARRIS, L. J., and WANG, Y. L. Vitamin methods. I. An improved procedure for estimating vitamin B_1 in foodstuffs and biological materials by the thiochrome test including comparisons with biological assays. Biochem. J. **35**: 1050–1070 (1941).
18. HAYWARD, J. W., STEENBOCK, H., and BOHSTEDT, G. The effect of heat as used in the extraction of soybean oil upon the nutritive value of the protein of soybean oil meal. J. Nutrition **11**: 219–234 (1936).
19. HENDERSON, L. M., and SNELL, E. E. A uniform medium for determination of amino acids with various microorganisms. J. Biol. Chem. **172**: 15–29 (1948).
20. HUBBELL, REBECCA, MENDEL, L. B., and WAKEMAN, A. J. A new salt mixture for use in experimental diets. J. Nutrition **14**: 273–285 (1937).
21. JONES, D. B., and WIDNESS, KATHERINE. The comparative growth-promoting value of the proteins of wheat germ, corn germ, and of some other protein foods of plant and animal origin. J. Nutrition **31**: 675–683 (1946).
22. KIRK, P. L., and MOBERG, E. G. Microdetermination of calcium in sea water. Ind. Eng. Chem. (Anal. Ed.) **5**: 95–98 (1933).
23. KIRK, P. L., and SCHMIDT, C. L. A. An improved technique for micro calcium analysis. J. Biol. Chem. **83**: 311–314 (1929).
24. KOENIG, RUTH, and JOHNSON, C. R. Spectrophotometric determination of iron. II. Use of 2, 2′-Bipyridine. J. Biol. Chem. **143**: 159–163 (1942).
25. MENDEL, L. B., and FINE, M. S. Studies in nutrition. IV. The utilization of the proteins of the legumes. J. Biol. Chem. **10**: 433–458 (1912).
26. MILLER, C. D. Thiamin content of Japanese soybean products. J. Am. Dietet. Assoc. **21**: 430–432 (1945).
27. MITCHELL, H. H., HAMILTON, T. S., and BEADLES, J. R. The importance of commercial processing for the protein value of food products. J. Nutrition **29**: 13–25 (1945).
28. OSBORNE, T. B., and MENDEL, L. B. The use of soybean as food. J. Biol. Chem. **32**: 369–376 (1917).
29. PAYNE, D. S., and STUART, L. S. Soybean protein in human nutrition. Advances in protein chemistry, Vol. I. Academic Press: New York (1944).
30. PETERSON, W. J., BRADY, D. E., and SHAW, A. O. Fluorometric determination of riboflavin in pork products. Ind. Eng. Chem. (Anal. Ed.) **15**: 634–636 (1943).
31. SCHROEDER, L. J., CAHILL, W. M., and SMITH, A. H. The utilization of calcium in soybean products and other calcium sources. J. Nutrition **32**: 413–422 (1946).
32. SHERMAN, W. C., ELVEHJEM, C. A., and HART, E. B. Further studies on the availability of iron in biological materials. J. Biol. Chem. **107**: 383–394 (1934).
33. SNEDECOR, G. W. Statistical methods. Collegiate Press: Ames, Iowa (1942).
34. SPRAGUE, P. E. Edible soy flour. Soy Flour Association: Chicago, Illinois (1940).
35. TEPLY, L. J., STRONG, F. M., and ELVEHJEM, C. A. The distribution of nicotinic acid in foods. J. Nutrition **23**: 417–423 (1942).

EFFECT OF BISULFITE, ACETALDEHYDE, AND SIMILAR REAGENTS ON THE PHYSICAL PROPERTIES OF DOUGH AND GLUTEN [1]

I. HLYNKA

ABSTRACT

Small amounts of sodium bisulfite produce a soft, sticky, and inelastic gluten; larger amounts destroy the gluten-yielding property of dough. Acetaldehyde can prevent bisulfite from affecting the gluten when the two reagents are incorporated simultaneously into dough, but it can also counteract existing effects in bisulfite-treated gluten. By means of bisulfite-acetaldehyde treatment some inferior dried glutens, which could not be reconstituted with water alone, were reconstituted to glutens of reasonably good physical quality. A large number of common reducing agents were found ineffective in producing in dough an effect similar to that of bisulfite. Bisulfite-like reactions were produced with cysteine, glutathione, and sulfide. But various differences occurred in effects of the reagents on gluten, and in the modification of effects caused by acetaldehyde. The results are discussed from the point of view of dissociation and formation of cross-linkages in a three-dimensional network of gluten. Either the disulfide may provide the necessary cross-linkages or carbonyl compounds may act as cross-linking agents between protein molecules.

Although elasticity is one of the basic and most characteristic properties of dough and gluten, our knowledge of its structural basis is meager. We know that the requisites for elasticity in polymers are, first, a long chain, and second, cross-linkages joining the linear polymer chains at intervals to form a three-dimensional network. The gluten proteins in dough undoubtedly provide the linear polymer in the polypeptide structure. However, little experimental evidence exists as to the identity of the cross-linkages, although the disulfide linkages are assumed to be involved (5, 11).

The most pertinent work on the disulfide cross-linkages in proteins has come from the laboratories of the Wool Industries Research Association (3). It has been shown that overnight treatment of wool with bisulfite (usually 13%), at room temperature, breaks the disulfide linkage to form a free thiol group (R-SH) and perhaps an unstable sulfenic acid (R-SOH) which adds sulfite to from cysteinyl S-sulfonate ($RSSO_2ONa$). Lindley (7) treated gliadin with a 25% sodium sulfite

[1] Manuscript received December 30, 1948.
Paper No. 96 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 266 of the Associate Committee on Grain Research (Canada).

solution at room temperature overnight and obtained a fractionation similar to that for wool.

An alternate possibility is suggested by the work of Fraenkel-Conrat and Olcott (4) which showed that aldehydes can act as cross-linking agents between amide, amino, and guanidyl groups, on the one hand, and ammonia, primary amines, and amino acids, on the other. Gliadin treated with formaldehyde was found to bind large amounts of ammonia, amines, and amino acids. This information becomes more significant when considered together with the well-known tendency of carbohydrates to react, through the carbonyl group, with proteins in the so-called browning reaction. In other words, reducing carbohydrates may conceivably act as cross-linking agents in dough.

If it is assumed that the structural basis of elasticity in dough relates to cross-linkages between protein chains, it may be inferred that reagents that change the elasticity do so either by establishing or dissociating cross-linkages, or by splitting protein chains, i.e., by affecting protease activity. On these grounds, the chemists' chief problem appears to be that of elaborating hypotheses at the molecular level which are consistent with observations made on the properties of the dough and on the changes in these properties caused by certain reagents. Among the more interesting reagents that affect dough properties are bisulfite and acetaldehyde. Small amounts of bisulfite produce a soft, sticky, and inelastic dough; acetaldehyde reverses the effect produced with bisulfite. The influence of these and other closely related reagents on dough and gluten is described in this paper and discussed in terms of hypotheses relating to the molecular reactions that may be involved.

Methods and Results

The Effect of Bisulfite and Acetaldehyde on Dough and Gluten. Small amounts of bisulfite markedly modify the elasticity of dough and gluten and the behavior of dough in the gluten-washing process. Changes in these properties were therefore used in assessing the effect of adding bisulfite and acetaldehyde to dough or gluten.

Gluten-washing experiments according to the A.O.A.C. method (1) were carried out on a casual sample of flour in which 10 mg. sodium bisulfite per 25 g. flour were incorporated into the dough. This amount of bisulfite was sufficient to destroy the gluten-yielding property of dough when washed in the usual way by hand; i.e., no elastomer was formed. In parallel experiments in which various amounts of acetaldehyde solution were added simultaneously with bisulfite, elastic gluten was readily obtained. The minimum amount of acetaldehyde required to prevent the action of 10 mg. bisulfite in dough was approximately 10 mg. On a mole-equivalent basis the ratio is 2.4 to 1.

Variations of the above experiments showed that soft, sticky, and inelastic gluten could be obtained by washing bisulfite-treated dough in a beaker with a stirring rod under a stream of cold water. This gluten completely recovered its normal handling characteristics and elasticity upon resting for an hour or two in water containing acetaldehyde up to 0.5%. Again, good gluten obtained in a normal way deteriorated rapidly in quality when placed in a solution of bisulfite, but recovered when subsequently placed in an acetaldehyde solution. Alone, acetaldehyde had a slightly toughening effect on gluten. The various reactions could be speeded up either by working the gluten with a stirring rod or by pinching off small pieces into a solution containing the reagent. Moreover, the effects of successive treatments with bisulfite and acetaldehyde have been demonstrated several times on the same sample of gluten.

Thus, acetaldehyde not only prevents bisulfite from affecting gluten when the two reagents are incorporated simultaneously into dough, but it also counteracts effects already caused by bisulfite. This observation was sufficiently new and interesting in itself to warrant further exploration. In addition, through the mechanism of the bisulfite and acetaldehyde reactions, information was sought on the identity of the cross-linkages which are responsible for the elastic property of dough and gluten.

Bisulfite and Acetaldehyde Experiments with Deteriorated Gluten. Treatment with bisulfite and acetaldehyde produced unusual results with samples of dried gluten which had deteriorated with age. Several preparations of varying quality and history were treated as follows: Five-gram amounts were reconstituted with water and allowed to rest for one hour. They were then treated for one hour with bisulfite alone, or with acetaldehyde alone. In addition, some samples were treated with bisulfite and one hour later with acetaldehyde and examined at the end of another hour. All samples were worked several times during the course of the treatments. The amounts of reagents were 1 ml. of a solution containing 5 mg. bisulfite per ml. and 2 ml. of 5% acetaldehyde solution. Results shown below were selected for greatest response to the bisulfite-acetaldehyde treatment; data on samples showing no response are omitted. Zero rating was assigned to gluten which formed only a "mush" and +++ to good, elastic gluten.

Gluten No.	*Treatment*			
	Water	*Bisulfite*	*Acetaldehyde*	*Bisulfite-acetaldehyde*
1	0	0	+	+++
2	0	+	+	+++
3	0	+	+	+++

It is remarkable that glutens which rated zero when reconstituted with water alone were brought to a +++ rating with the bisulfite-acetaldehyde treatment. This improvement, however, was not permanent and the glutens reverted to their impaired condition on continued washing under the tap.

The Effect of Reagents Possessing Action Similar to Bisulfite and Acetaldehyde. Substances containing the sulfhydryl group have been shown to affect dough and gluten in a way similar to bisulfite (2, 7, 10, 11). Preliminary comparative experiments were carried out with cysteine, glutathione, and sodium sulfide, and the combined effects of acetaldehyde and each of these reagents were also determined.

When 25 mg. of cysteine hydrochloride were incorporated into dough (25 g. flour) no gluten could be recovered by the usual washing procedure. With smaller concentrations, sticky, soft, and inelastic gluten was obtained. This gluten, however, showed some improvement in physical properties with continued washing (an effect which was not apparent with bisulfite-treated gluten). The action of cysteine was inhibited by simultaneous addition of acetaldehyde.

When normal gluten from 25 g. flour was placed in 75 ml. water containing 25 mg. cysteine hydrochloride, its surface soon became gelatinous. After several hours, the gluten became soft, inelastic, and sticky. This impaired gluten recovered its physical properties when subsequently placed in water containing 1 ml. of 10% acetaldehyde solution. A slight improvement was also obtained in water alone.

Similar experiments were made with glutathione. Increments up to 40 mg. were incorporated with 25 g. flour into dough. Reduced yields of impaired and sticky gluten were obtained. This, like cysteine-treated gluten, showed a slight improvement on prolonged washing. However, addition of acetaldehyde simultaneously with glutathione gave no recovery of gluten by washing; in this respect glutathione is dissimilar to cysteine.

Gluten placed into 75 ml. water containing 40 mg. glutathione became slippery and glutinous on the surface and after several hours became soft, inelastic, and sticky. This impaired gluten showed slight recovery in water but disintegrated rapidly in dilute solutions of acetaldehyde. Here, again, glutathione is dissimilar to cysteine.

Disintegration was also noted in comparative experiments with papain in which greater destruction was obtained in doughs containing acetaldehyde and papain than in those containing papain alone.

Sodium sulfide was required in relatively large amounts to produce effects similar to those of cysteine and glutathione. Dough made from 25 g. of flour with 150 mg. of sodium sulfide still gave recovery of a weakened gluten, which seemed to improve with washing. When small

pieces of normal gluten were pinched off into a 1% buffered solution of sodium sulfide, the gluten became very slippery, a great deal seemed to disperse, and only a small amount of inelastic, sticky gluten could be gathered together. This impaired gluten recovered almost entirely in acetaldehyde solutions, but only slightly, if at all, in water.

The effect on bisulfite-treated dough and gluten of several substances which were expected to have the same action as acetaldehyde was also ascertained. Formaldehyde, acetone, glucose, and wheat starch were found to possess some activity in preventing or reversing the effect of bisulfite on gluten. Acetaldehyde was approximately twice as active as formaldehyde and about 100 times as active as acetone when these reagents were incorporated into dough simultaneously with bisulfite. A small positive effect was also noted with glucose and wheat starch when they were first worked into bisulfite-impaired gluten and then washed out again.

The Action of Reducing Agents. Because the effect of bisulfite, cysteine, glutathione, and sulfide has been attributed to their reducing action (8, 10, 11), a number of other reducing agents were studied. The destruction of gluten-yielding ability of dough in the gluten-washing test already described was used as the basis of comparison. The reducing agents listed were tried in equimolar, 5 times, and 25 times the molar concentration of bisulfite required to destroy gluten in the gluten-washing test.

The following chemicals did not produce a similar effect to that of bisulfite as judged by the gluten-washing test. The gluten could be washed out normally; but at higher concentrations of some reagents, a short, harsh texture, rather than a soft, pliable, and smooth one, was obtained.

ascorbic acid
calcium metal
(50 mg./25 g. flour)
cuprous chloride
ferrous ammonium sulfate
ferrous chloride
ferrous sulfate
hydroquinone
mercurous chloride
neutral red
potassium ferrocyanide
pyrogallic acid
sodium thiosulfate
stannous chloride

Those chemicals which had a strong acid reaction, such as ascorbic acid and stannous chloride, were used in 15 ml. of phosphate buffer solution with which the dough was made up. Others were used in aqueous solutions.

A few values for standard oxidation-reduction potentials show that strongly reducing substances are included in the above list. For example, mercurous-mercuric system has an E_o value of -0.92 volts; ferrous-ferric, -0.74 volts; ferrocyanide-ferricyanide, -0.49 volts; and stannous-stannic, -0.13 volts. Since a variety of reducing agents

have no effect on gluten, the effect of bisulfite appears to involve some more specific reaction than mere reduction.

Farinograms and Extensograms on Doughs Treated with Bisulfite and Acetaldehyde. The criteria so far used in assessing the effects of vari-

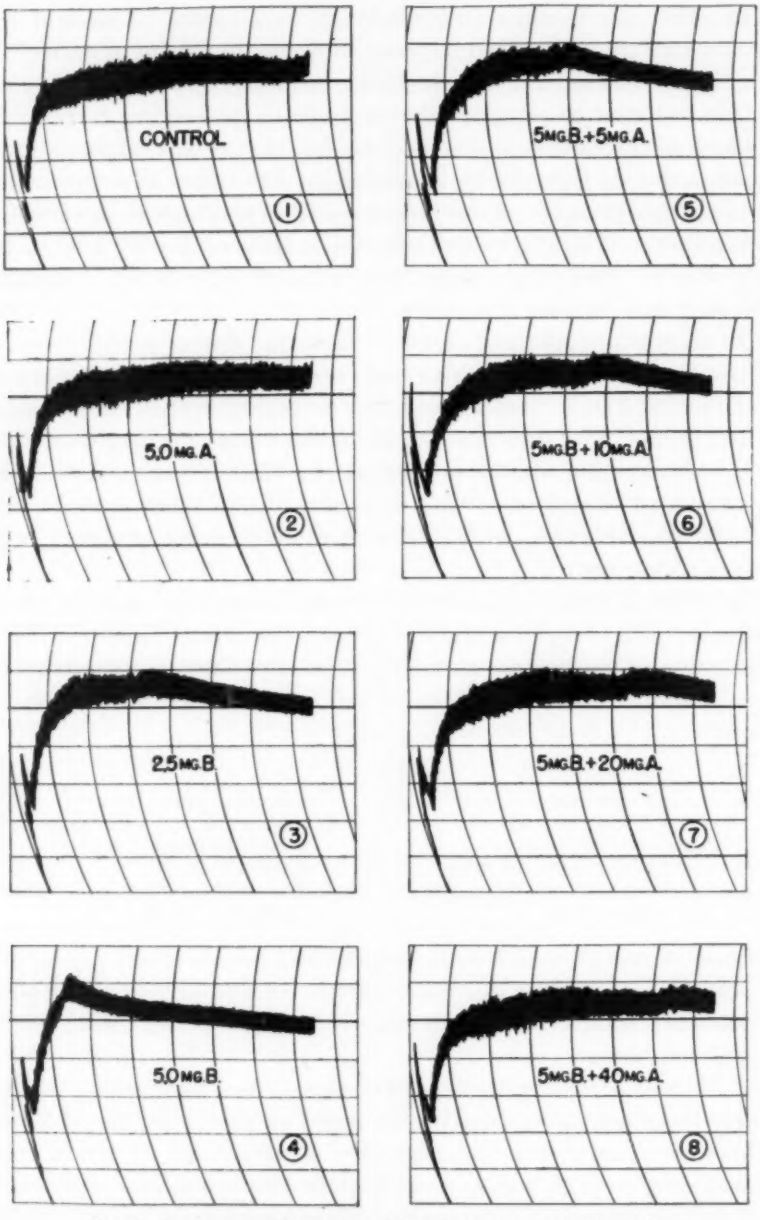

Fig. 1. Farinograms showing the effect of bisulfite and acetaldehyde on dough: B = bisulfite, A = acetaldehyde in mg.%.

ous reagents on dough and gluten involve subjective judgments which are not very precise. Accordingly, several experiments were carried out on the farinograph and extensograph which are capable of yielding data that are more precise and objective, though still empirical. Unfortunately, since it was not experimentally feasible to add acetaldehyde to the doughs after they were formed, no tests were conducted to show that the effect of bisulfite could be reversed with acetaldehyde. Experiments were therefore confined to tests in which the action of bisulfite in dough was merely prevented by the simultaneous addition

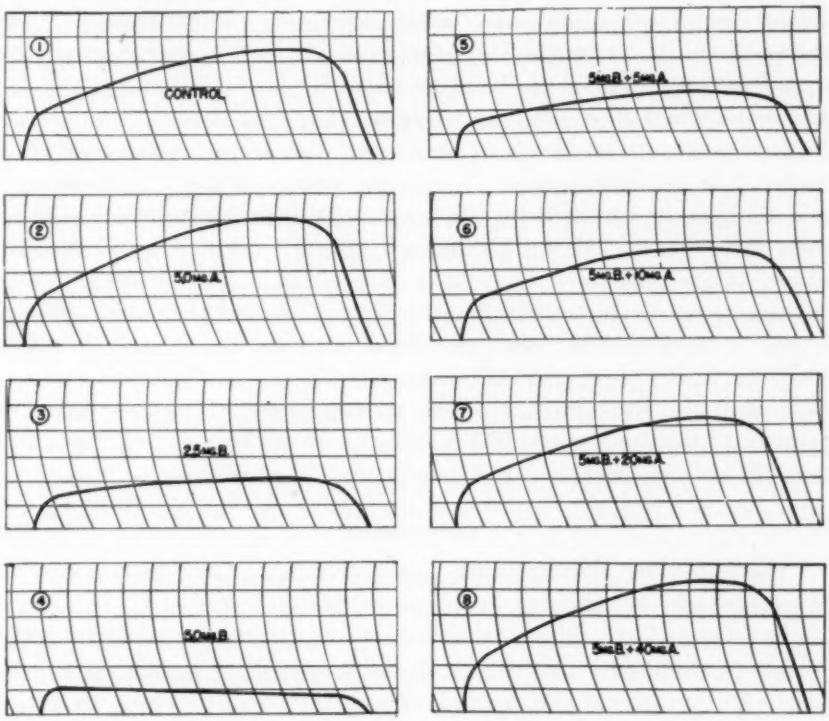

Fig. 2. Extensograms showing the effect of bisulfite and acetaldehyde on dough: B = bisulfite, A = acetaldehyde in mg.%.

of acetaldehyde. It should also be borne in mind that the farinograph and the extensograph measure physical properties of dough. Therefore, the results obtained previously in tests which depend on the gluten-yielding ability of dough when washed, and on the physical properties of gluten obtained, are not necessarily comparable with farinogram and extensogram results.

The effect of bisulfite and acetaldehyde on the physical properties of dough is shown by farinograms in Fig. 1. The strength of the flour represented by farinogram 1 is reduced successively by the effects of

2.5 and 5.0 mg.% bisulfite shown by farinograms 3 and 4. Farinogram 4 is typical of a flowy but inelastic dough such as is obtained from a soft wheat flour. Now, although acetaldehyde alone has no appreciable effect on the dough, as shown by farinogram 2, it can nullify the effect of bisulfite when the two reagents are added simultaneously; farinogram 5 in which the dough was treated with 5 mg.% bisulfite plus 5 mg.% acetaldehyde is approximately the same as farinogram 3 in which the dough contained only 2.5 mg.% bisulfite. From this comparison it may be estimated that approximately 4 moles of acetaldehyde is equivalent to 1 mole of bisulfite. This equivalence ratio is higher but of the same order of magnitude as that obtained by the gluten-washing technique. Farinograms 7 and 8 show practically complete inhibition of the bisulfite effect by acetaldehyde; the dough retained normal elasticity and handling characteristics.

Extensograms were also made with the same concentration of reagents as for farinograms. These are shown in Fig. 2. Resistance to extension, represented by the height of the extensogram, is reduced from 450 units to 200 and 100 units for the 2.5 and 5.0 mg.% bisulfite levels. Extensogram 4 is typical of extensible but inelastic dough, characteristic of soft wheat flour. Curve 7 shows that the action of 5 mg.% bisulfite has been prevented with 20 mg.% acetaldehyde. Curves 2 and 8 show that acetaldehyde has a small positive effect in increasing the resistance of dough to extension. It is interesting to point out also that practically no change in extensibility of the dough took place in the bisulfite and acetaldehyde treatments.

Discussion

The foregoing data establish that the damage from bisulfite action to gluten can be reversed with acetaldehyde, although these compounds do not form an oxidation-reduction system. Subsidiary to this finding is that only a restricted group of reducing agents have a bisulfite-like effect on dough, and that there are differences in action among bisulfite, cysteine, glutathione, and sodium sulfide. These topics will be briefly discussed, and their relation to hypotheses about cross-linkages as the structural basis of elasticity in dough and gluten will be indicated.

Firstly, the reversibility of bisulfite damage in gluten does not appear to be consistent with the hypothesis that proteolysis is involved, as has been suggested by Jørgensen (6) and by Balls and Hale (2). Secondly, the effect of bisulfite on gluten cannot be adequately explained in general terms of oxidation-reduction, since the bisulfite and acetaldehyde used in our studies do not constitute an oxidation-reduction system. This also follows from the finding that many re-

ducing agents do not have a bisulfite-like effect on dough. It appears that the effect is restricted to bisulfite, sulfhydryl compounds, and perhaps cyanide.

Certain differences in the effects on gluten produced by bisulfite, cysteine, glutathione, and sulfide suggest that more than one reaction is involved. Bisulfite produces a characteristically weak and very sticky gluten; sulfide produces gluten with a slippery gelatinous surface; and the remaining compounds produce effects intermediate between these two. The effect of acetaldehyde on glutens treated with different reagents also shows great differences. Acetaldehyde counteracts or reverses the effects of bisulfite, cysteine, and sulfide, but not those of glutathione or papain, with which a different reaction comes into prominence. At very small concentrations, acetaldehyde may partly reverse glutathione damage in gluten, but this reaction is obscured by a disintegrating action which becomes pronounced at concentrations of approximately 0.5%. Acetaldehyde has also a strong disintegrating effect on papain-treated gluten. Proteolytic mechanism for the effect of glutathione on gluten is therefore not excluded. Finally, there is a difference in reactivity of the different reagents. On a mole-equivalent basis, bisulfite is the most effective, followed by cysteine and glutathione, with sodium sulfide very much less effective.

We now come to an interpretation of these summarized findings in terms of the hypotheses set out in the beginning. So far it has been indicated that the loss of characteristic elasticity when gluten is treated with bisulfite cannot be explained on the basis of proteolysis. It has also been suggested that more than one reaction is involved in the action of bisulfite, cysteine, glutathione, and sulfide on gluten. Let us now consider to what extent these reactions can be identified.

Elsworth and Phillips (3) definitely showed that bisulfite dissociates disulfide linkages in wool. Patterson *et al.* (9) pointed out that wool is also susceptible to sulfhydryl compounds, sulfide, and cyanide, the same group to which gluten is susceptible. It may be inferred from studies on wool and from similar experiments on gliadin (7) that the action of bisulfite on gluten is on the disulfide linkages, and from the reported data on reducing agents that this reaction is disulfide fission rather than an oxidation-reduction reaction. Acetaldehyde thus appears to prevent or reverse the reaction by binding the bisulfite and removing it from the reacting system. Changes in elastic properties may thus be explained by fission and reconstitution of the disulfide cross-linkages. On this basis, a definite correlation would be expected between disulfide content of gluten and its elastic properties. No publication establishing that such a correlation exists has come to the author's attention.

The alternate or additional possibility of forming cross-linkages between carbonyl groups of reducing carbohydrates, on the one hand, and amine, amide, and guanidyl groups of proteins, on the other, must also be examined. It may not be altogether co-incidental that compounds possessing a bisulfite-like effect are also characterized by their ability of adding to carbonyl groups. They could thus free protein from combination in a protein carbohydrate-protein complex. Again, acetaldehyde would simply remove bisulfite from the reacting system by forming an addition compound with it. It is interesting in this connection that bisulfite, which is the most effective compound in preventing browning reaction between carbohydrates and proteins, is also most effective in its action on gluten properties. It is realized, of course, that the effectiveness of bisulfite in preventing browning reaction can be explained in other ways, but the above observation is none the less suggestive.

At this stage, there is insufficient evidence to exclude either theory of cross-linkages in gluten. It is likely that both play a part. Future work, however, may eventually determine the part that these or other reactions play in the changes that occur in the physical properties of dough.

Literature Cited

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 6th ed. A.O.A.C.: Washington (1945).
2. Balls, A. K., and Hale, W. S. Further studies on the activity of proteinase in flour. Cereal Chem. **13**: 656–664 (1936).
3. Elsworth, F. F., and Phillips, H. The action of sulphites on the cystine disulphide linkages in wool. I. The influence of pH value on the reaction. Biochem. J. **32**: 836–843 (1938).
4. Fraenkel-Conrat, H., and Olcott, H. S. The reaction of formaldehyde with proteins. V. Cross-linking between amino and primary amide or guanidyl groups. J. Am. Chem. Soc. **70**: 2673–2684 (1948).
5. Halton, P., and Scott Blair, G. W. The relationship between conditions governing rupture and flow in flour doughs. J. Phys. Chem. **40**: 811–819 (1936).
6. Jørgensen, H. Studies on the nature of the bromate effect. Einar Munkagaard: Copenhagen (1945).
7. Lindley, H. The reactivity of the combined cystine of proteins other than wool. Biochem. J. **42**: 481–485 (1948).
8. Olcott, H. S., Sapirstein, L. A., and Blish, M. J. Stability of wheat gluten dispersions towards reducing agents in the presence and absence of a gluten proteinase. Cereal Chem. **20**: 87–97 (1943).
9. Patterson, W. I., Geiger, W. B., Mizell, L. R., and Harris, M. Role of cystine in the structure of the fibrous protein, wool. J. Research Nat. Bur. Standards **27**: 89–103 (1941).
10. Sandstedt, R. M., and Fortmann, K. The relation of proteolysis to the characteristics of oxidation and reduction in doughs. Cereal Chem. **20**: 517–528 (1943).
11. Sullivan, B., Howe, M., Schmalz, F. D., and Astleford, G. R. The action of oxidizing and reducing agents on flour. Cereal Chem. **17**: 507–528 (1940).

EFFECT OF COMMERCIAL FERTILIZERS AND GREEN MANURE ON YIELD AND NUTRITIVE VALUE OF WHEAT [1]

I. NUTRITIVE VALUE WITH RESPECT TO TOTAL PHOSPHORUS, PHYTIC PHOSPHORUS, NONPHYTIC PHOSPHORUS, AND CALCIUM CONTENT OF THE GRAIN

G. S. BAINS [2]

ABSTRACT

Studies were made on the effect of potassium nitrate, superphosphate, and superphosphate plus ammonium sulfate in doses of 25 lb. phosphoric acid (P_2O_5) and 60 lb. nitrogen per acre with and without green manure on the yield and nutritive value of wheat. The phosphatic treatments increased the yield by 38 to 48% over the control. As the phytic phosphorus was found to be positively correlated with calcium content ($r = +0.74$), the nonphytic phosphorus content was taken as the criterion of nutritive value. The percentage proportion of nonphytic phosphorus to total phosphorus ranged from 23.4% in grain of plots fertilized with superphosphate plus ammonium sulfate and green manure to 37.0% in grain of the plots treated with superphosphate plus green manure. The availability of phosphorus as determined by *in vivo* experiments, however, exceeded the corresponding nonphytic phosphorus values by 27 to 32%.

The importance of calcium and phosphorus in nutrition is a well-recognized fact. The distribution of various minerals, inclusive of calcium and phosphorus, in plants, vegetables, and food grains and the effect of various factors, such as climate, irrigation, soil, and manures, on the composition of food products with particular reference to the total phosphorus and calcium content in grains has been investigated rather exhaustively. Greaves and Hirst (6) reported great variation in the calcium and phosphorus content of wheat grains due to differences in variety, soil type, and irrigation. Murphy (17) observed that manuring with superphosphate increased the total phosphorus content of wheat.

Observations of similar nature have also been reported in the case of forage crops. It is well known that phosphatic fertilizers cause a marked increase in the total phosphorus content of crops and plants

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grown on phosphate-deficient soils, but accurate information is lacking as to whether this increase is in organic or inorganic form and how such variations are associated with the calcium content of the grains. Numerous workers (3, 4, 10, 16, and 22) demonstrated the beneficial effect of green manuring in releasing and making available calcium and iron to the plants from the bound and unavailable forms. However, there is little information concerning the effect of green manure applied in conjunction with artificial fertilizers, especially those containing phosphorus, on the total phosphorus and calcium content of the grains; the effect of such a combination on calcium and on the various forms of phosphorus present in the grain has not been studied.

Considerable literature is extant to show that estimations of calcium and phosphorus content of the grain may be an unreliable index of the value of grain as a source of these elements.

There are a number of organic phosphorus compounds, such as phytin, phospholipids, hexose-phosphates, nucleic acid, and phosphoproteins, etc., which occur in plants and plant seeds. Of these, phytin is of considerable interest. Posternak (20) observed that phytin phosphorus constituted 70 to 90% of the total phosphorus of the wheat grain. His observations have been corroborated by the work of Andrews and Bailey (2), Knowles and Watkin (11), and Harris and Mosher (7), that some 40 to 70% of the total phosphorus in cereal grains is present in the form of phytin. Recent investigations throw considerable light on the probable role of phytin in human nutrition. A comprehensive account of the nature and significance of this compound has been given by Harrison and Mellanby (8), who stated that phytic acid renders calcium unavailable. In a rachitogenic cereal, phytic acid immobilizes all or almost all of the calcium contained in the cereal by converting it into insoluble calcium phytate, which is not absorbed, while the excess over and above that required to precipitate the calcium of the cereals can exert an additional anticalcifying action by precipitating further amounts of the calcium from the noncereal part of the diet. This view fits in with the observations that the anticalcifying effect of the cereals can be antagonized by feeding extra calcium. In some earlier investigations, Mellanby (13, 14) found that the addition of calcium salts, such as calcium carbonate and calcium phosphate, to a diet largely composed of oat meal reduces its rachitogenic effect. These results have since been confirmed by Mottram and Palmer (15, 18) by using calcium lactate.

Cereals constitute a major portion of the diet of millions of people in India. The consumers, for want of adequate milk supply, mainly depend upon them for their requirements of essential minerals such as calcium and phosphorus. The importance of cereals as the main source

of these elements assumes greater significance in view of the acute shortage of food grains in the country, especially when it is also known that a greater portion of the total phosphorus of the grain is present in the relatively unavailable phytic form. The extent to which commercial fertilizers in conjunction with green manure can increase yield and improve the nutritive value of wheat, which is one of the major food crops of the country, was, therefore, considered worth while to investigate. Besides recording yield data, determinations were made of the calcium, total phosphorus, and phytic and nonphytic phosphorus content of the grain. *In vivo* studies have also been carried out to find out the influence of variations in the phosphorus content of the grain, due to fertilization, on the availability of this mineral by the "balance sheet" method.

Material and Methods

The material employed in the present investigation consisted of wheat C 409 (1938–39) crop sown under field conditions and was obtained from the replicated plots of the four continuous randomized blocks of the manurial experiments underway at the Agricultural Experimental Station, Rawalpindi. This Experimental Station is situated in the northwest part of the Punjab, in a submountainous tract. The soil of this farm is deficient in available phosphates and shows a remarkable response to the application of phosphatic manures, especially superphosphate. Representative samples of wheat were collected from plots fertilized as shown in Table I.

There were eight treatments, each in quadruplicate. The size of each plot was 1/40th of an acre. Representative samples were collected separately from all of these plots, and were freed from dust and foreign matter. The required quantities were ground to a uniformly fine meal, thoroughly mixed to obtain a homogeneous sample, and

TABLE I

AMOUNTS OF FERTILIZER APPLIED PER ACRE

No.	Fertilizer treatment	Nitrogen as N_2	Phosphorus as P_2O_5	Green manure
		lb.	*lb.*	*tons*
1	Control (No manure)	0.0	0.0	0.0
2	Green manure (*Cajanus indicus*)	0.0	0.0	5.0
3	Potassium nitrate	60.0	0.0	0.0
4	Potassium nitrate, green manure	60.0	0.0	5.0
5	Superphosphate	0.0	25.0	0.0
6	Superphosphate, green manure	0.0	25.0	5.0
7	Superphosphate, ammonium sulfate	60.0	25.0	0.0
8	Superphosphate, ammonium sulfate, green manure	60.0	25.0	5.0

preserved in airtight bottles. The samples from each plot were analyzed for various chemical constituents.

Total phosphorus was determined by the standard A.O.A.C. method of estimating phosphorus volumetrically. Phytic phosphorus was determined by the procedure of McCance and Widdowson (12) with the modification suggested by Snook (21). The modification consisted in using 2 ml. of concentrated sulfuric acid instead of a mixture of sulfuric acid and perchloric acid for the wet digestion of sodium phytate. Nonphytic phosphorus was obtained by subtracting the amount of phytic phosphorus from the total phosphorus.

Calcium was estimated in accordance with the A.O.A.C. volumetric method involving precipitation as calcium oxalate and titration with standard potassium permanganate solution.

In the *in vivo* studies, the technique based on the "balance sheet" method as used by Henry and Kon (9) and Giri (5) was adopted. Five groups of young albino rats, weighing 60 to 70 g., each group having two males and two females, were placed in metabolism cages. The basic portion of the diet fed to the animals was composed of the following parts: dried egg white powder, 14; salt mixture (phosphorus and calcium-free), 3; powdered sugar, 10; coconut oil, 5; and cod liver oil, 3. In addition pure thiamine (3γ) was given daily along with the food. The cereal constituted 75% of the diet. The rats were fed with weighed quantities of the diets, which were in excess of the normal requirements of the different groups. The residues were collected daily and dried in an electric air oven at 100°C. After a preliminary period of 3 days, feces and urine were collected daily for a period of 14 days. The urine of each group was mixed together, carefully concentrated on a water bath, dried on a sand bath, and the phosphorus determined volumetrically after ashing a known amount. Similarly, dry feces voided by each group were mixed, powdered, and passed through a fine mesh sieve. A weighed amount, 10–12 g., was ashed in a platinum dish and the phosphorus in the ash determined volumetrically.

Results and Discussion

Effect of Fertilization on Yield. The mean yields of wheat grain obtained from plots given different fertilizer treatments are shown in Table II. The application of various fertilizers to plots sown with wheat appreciably enhanced the yield of grain in all cases. There is a marked increase (38–48%) in the yield of grain of plots receiving superphosphate in various combinations as compared with the yield of grain from control plots. The addition of green manure along with the artificial fertilizers did not materially increase the yield above that

TABLE II

EFFECT OF FERTILIZATION ON YIELD OF WHEAT

No.	Fertilizer treatment	Mean yield per acre	Mean yield of main treatments, per acre	Mean yield of main treatments, as per cent of control	Increase or decrease due to green manuring
		lb.	*lb.*	%	%
1	Control (No manure)	664.0	700.0	100.0	+10.8
2	Green manure	736.0			
3	Potassium nitrate	873.6	831.6	118.8	− 9.6
4	Potassium nitrate, green manure	789.6			
5	Superphosphate	992.0	1038.0	148.2	+ 9.3
6	Superphosphate, green manure	1084.0			
7	Superphosphate, ammonium sulfate	928.0	970.0	138.2	+ 9.1
8	Superphosphate, ammonium sulfate, green manure	1012.0			

obtained with artificial fertilizers alone. While the yield was increased by about 9.1 to 10.8% by green manure in plots receiving fertilizer treatment Nos. 2, 6, and 8, they were lower by about 9.6% in the case of potassium nitrate applied with green manure. It may be that in the bacterial decomposition of green manure some of the nitrates might have been consumed.

TABLE III

EFFECT OF FERTILIZATION ON TOTAL PHOSPHORUS, PHYTIC PHOSPHORUS, NONPHYTIC PHOSPHORUS, AND CALCIUM CONTENT OF WHEAT

(Analytical data expressed on moisture-free basis)

No.	Fertilizer treatment	Phosphorus per 100 g.			Non-phytic[1] P	Phytic[1] P	Calcium per 100 g.
		Total	Phytic	Non-phytic			
		mg.	*mg.*	*mg.*	%	%	*mg.*
1	Control (No manure)	243.5	170.8	72.7	29.9	70.1	70.4
2	Green manure	272.2	179.6	92.6	34.0	66.0	70.5
3	Potassium nitrate	243.6	178.5	65.1	26.7	73.3	71.5
4	Potassium nitrate, green manure	263.3	180.7	82.6	31.4	68.6	72.0
5	Superphosphate	276.7	191.7	85.0	30.7	69.3	71.2
6	Superphosphate, green manure	316.3	199.4	116.9	37.0	63.0	73.8
7	Superphosphate, ammonium sulfate	299.8	222.6	77.2	25.7	74.3	82.8
8	Superphosphate, ammonium sulfate, green manure	264.5	202.7	61.8	23.4	76.6	74.9

[1] Phytic and nonphytic phosphorus as per cent of total phosphorus.

Effect of Fertilization on Chemical Composition of Grain. The results of chemical analyses of the wheats are given in Table III. The total phosphorus and phytic phosphorus content of the grain obtained from plots to which superphosphate was applied in various combinations (treatment Nos. 5, 6 and 7, 8) was higher than the corresponding values of the grain obtained from control and potassium nitrate-treated plots considered together. The mean total and phytic phosphorus of wheat obtained from plots receiving superphosphate (treatment Nos. 5, 6 and 7, 8) was 289.3 and 204.1 mg./100 g. as compared with 255.6 and 177.4 mg./100 g. for the grain from plots which did not receive superphosphate (treatment Nos. 1, 2 and 3, 4). The calcium content of wheat grain obtained from plots receiving phosphatic treatments, with the exception of treatment No. 5, was also higher than that of the grain from nonphosphatic treatments. Total phosphorus and nonphytic phosphorus content of the grain which received superphosphate and green manure was the highest, i.e., 316.3 and 116.9 mg./100 g., respectively, while the grain obtained from plots treated with superphosphate in conjunction with ammonium sulfate showed the highest content of phytic phosphorus and calcium, i.e., 222.6 and 82.8 mg./100 g., respectively.

The application of various fertilizers with green manure, with the exception of treatment No. 8, produced grain with comparatively high contents of calcium and total phosphorus (both phytic and nonphytic). The combination of green manure with superphosphate and ammonium sulfate, however, decreased these constituents in the grain. The readily available nonphytic phosphorus constituted 23.4 to 37.0% of the total phosphorus of the grain. The percentage proportion of phytic phosphorus to total phosphorus was the lowest (63.0%) in the grain from plots treated with superphosphate and green manure, and was the highest (76.6%) in the grain from treatment No. 8. From these results it can be tentatively concluded that combination of ammonium sulfate with superphosphate is conducive to the formation of more phytic phosphorus in the grain.

Relationship between the Calcium and Phytic Phosphorus Content of the Grain. In view of the possible antagonizing action of phytic acid on the absorption of calcium in the system, it was of interest to know if there was any association between the calcium and phytic phosphorus contents of the various samples. The coefficient of correlation between these variables was calculated from the results of 32 individual samples obtained from the various fertilizer treatments and found to be $r = +0.74$, a value which was highly significant. The relative nutritive value with respect to calcium and phytic phosphorus may, therefore, not have been much influenced by the different

manurial treatments, since an increase in the phytic phosphorus content of the grain is usually accompanied by a corresponding increase in the calcium content.

Effect of Fertilization on the Biological Availability of Phosphorus. The nonphytic phosphorus content of the wheat samples may be regarded as more indicative of the nutritive value with respect to available phosphorus. As not more than 23.4 to 37.0% of the total phosphorus content of the wheats appeared to be in the available form, it was considered desirable to determine the relation between available phosphorus content of the grain and the results of biological assays for a few typical composite samples of wheat representing different manurial treatments. The results for the percentage of biologically available phosphorus on the basis of total phosphorus for the total period of the experiment are given in Table IV.

TABLE IV

TOTAL INTAKE, EXCRETION, AND BALANCE OF PHOSPHORUS RETAINED BY RATS FED ON VARIOUS DIETS CONTAINING 75% OF WHEAT GROWN ON PLOTS WHICH HAD RECEIVED DIFFERENT FERTILIZERS

Rat group[1] No.	Fertilizer treatment	Intake	Excretion			Phosphorus retained	Availability[2]	Ratio[3] of phytic to nonphytic, P
			Urinary	Fecal	Total			
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	%	
I	Control (no manure)	223.4	25.1	70.8	95.9	127.5	57	2.34
II	Superphosphate	303.5	25.3	85.8	111.1	192.4	63	2.25
III	Superphosphate, green manure	295.2	23.5	78.7	102.2	193.0	65	1.70
IV	Superphosphate, ammonium sulfate	250.1	21.9	82.5	104.4	145.7	58	2.88
V	Superphosphate, ammonium sulfate, green manure	237.6	30.5	78.6	109.1	128.5	54	3.28

[1] There was not much difference in the growth of the various groups.
[2] Availability as percentage of total phosphorus retained by the animals.
[3] Calculated by dividing the values of phytic phosphorus by the corresponding values of nonphytic phosphorus of the parent wheat samples (Table III).

The biological availability of the phosphorus of the different wheat samples ranged from 54 to 65%. The former figure (54%) was obtained for the wheat samples obtained from plots treated with superphosphate, ammonium sulfate, and green manure, while the latter (65%) for the wheat samples obtained from plots treated with superphosphate and green manure. On the basis of the nonphytic phosphorus content, as revealed by chemical analysis, the available phosphorus should constitute only 23.4 to 37.0% of the total phosphorus. Collating this with the values obtained for biologically available phosphorus it is evident that roughly 27 to 32% of the phytic phos-

phorus of the wheat samples was also available to the rats. The exact nature of the different forms in which phosphorus exists in the cereals is still obscure, but certainly a major portion of it exists as phytin, a calcium and magnesium salt of phytic acid. This compound is susceptible to enzymic decomposition. Patwardhan (19) showed that an enzyme capable of hydrolyzing phytin is present in the intestines of the albino rats. This enzyme may bring about a partial conversion of the organic phosphorus into an inorganic form, thus rendering a part of it available to the system. McCance and Widdowson (12) determined the actual amount of phytic phosphorus in the feces after adding phytin to the diet of human beings. They found that 20 to 60% of the phytin was excreted unchanged in the feces and suggested that much of the remaining phosphorus may also have remained unabsorbed and excreted through the feces in some other form. On the other hand, Anderson (1) isolated crystalline inositol monophosphate from an organic phosphorus fraction of wheat bran, which had resisted breakdown into inorganic phosphorus even after preserving it for two years.

It may also be possible that phytic phosphorus is not completely digested in the stomach and some of it passed out undigested. This explanation appears to be plausible, since in the laboratory 0.5 *N* hydrochloric acid solution is used to extract this form of phosphorus from the test material, whereas the strength of this acid in normal human gastric juice is approximately 0.1 *N*. Acid of the latter strength can extract only small amounts of the phytic phosphorus. It is, however, possible that the enzyme pepsin, which is also present in the gastric juice, may exercise some influence on its digestion in the stomach. The latter point was investigated by carrying out an *in vitro* digestion of a sample of wheat meal with 0.1 *N* hydrochloric acid solution alone and with the addition of pepsin. The extraction of phytic phosphorus with 0.1 *N* hydrochloric acid was 56.3%, but with a combination of pepsin and 0.1 *N* hydrochloric acid it was 86.8%. It is likely that extraction under natural conditions may be still more efficient. In the light of this observation, the view that phytic phosphorus may not be extracted completely from the grain complex in the stomach is not correct. However, the phytic phosphorus may also be regarded as present in some loose combination with the proteins of the grain, as a fair amount of it was discovered to have been released on enzymic digestion.

The differences in the percentage of biologically available phosphorus among the tested samples seem to bear some relation to their phytic and nonphytic phosphorus content. On comparing the figures for biologically available phosphorus and the ratio of phytic phosphorus to nonphytic phosphorus (Table IV) there appears to be

negative correlation between the two. The maximum value for biologically available phosphorus was obtained with wheat which had received superphosphate and green manure. Addition of green manure to the plots receiving superphosphate and ammonium sulfate decreased the total and nonphytic phosphorus content of the grain (Table III) as well as the percentage of biologically available phosphorus, which was the lowest in case of this treatment.

Literature Cited

1. ANDERSON, R. J. Concerning the organic phosphoric acid compound of wheat bran. III. Inositol monophosphate, a new organic phosphoric acid occurring in wheat bran. J. Biol. Chem. **18**: 441–446 (1914).
2. ANDREWS, J. S., and BAILEY, C. H. Distribution of organic phosphorus in wheat. Ind. Eng. Chem. **24**: 80–82 (1932).
3. BLACKSHAW, G. N. Green manuring and soil management. Rhodesia Agr. J. **18**: 455–460 (1921).
4. COLBOURN, H. J. Analyses of Tasmania soils. Agr. and Stock Dept. Tasmania. Bull. **33**: 18–33 (1916–1917).
5. GIRI, K. V. The availability of calcium and phosphorus in cereals. Indian J. Med. Research **28**: 101–111 (1940).
6. GREAVES, J. E., and HIRST, C. T. The mineral content of grain. J. Nutrition **1**: 293–298 (1929).
7. HARRIS, R. S., and MOSHER, L. M. Estimation of phytin phosphorus. Ind. Eng. Chem. (Anal. Ed.) **6**: 320–321 (1934).
8. HARRISON, D. C., and MELLANBY, E. Phytic acid and the rickets-producing action of cereals. Biochem. J. **33**: 1660–1680 (1939).
9. HENRY, K. M., and KON, S. K. Milk and nutrition. I. The effect of commercial pasteurization on the nutritive value of milk as determined by laboratory experiment. Raw and commercially pasteurized milks as sources of calcium and phosphorus for the growing rat. Natl. Inst. Research Dairying, Reading and Rowett Research Inst., Aberdeen. Unnumbered Pub. 9–31 (1937).
10. JENSEN, C. A. Effect of decomposing organic matter on the solubility of certain inorganic constituents of the soil. J. Agr. Research **9**: 253–268 (1917).
11. KNOWLES, F., and WATKIN, J. E. The amounts and distribution of some phosphorus and nitrogen compounds in wheat during growth. J. Agr. Sci. **22**: 755–766 (1932).
12. MCCANCE, R. A., and WIDDOWSON, ELSIE M. Phytin in human nutrition. Biochem. J. **29**: 2694–2699 (1935).
13. MELLANBY, E. Experimental rickets. Effects of cereals and their interaction with other factors of diet and environment in producing rickets. Med. Research Council (Brit.) Special Rept. Ser. No. 93 (1925).
14. MELLANBY, MAY T. Diet and teeth: an experimental study. 3 V. Med. Research Council (Brit.) Special Rept. Ser. No. 140, 153, 191 (1929–1934).
15. MOTTRAM, J. C., and PALMER, N. On the factors determining rickets in rats fed on cereal diets. Cereal Chem. **14**: 682–686 (1937).
16. MURPHY, E. W. Instinctive choice of food and water by stock. J. Dept. Agr., Victoria **16**: 55–56 (1918).
17. MURPHY, H. F. Effect of fertilizers on the yield and composition of wheat. J. Am. Soc. Agron. **22**: 765–770 (1930).
18. PALMER, N., and MOTTRAM, J. C. The influence of phytin and of fats on the production of rickets by a cereal diet. Biochem. J. **33**: 512–515 (1939).
19. PATWARDHAN, V. N. The occurrence of a phytin-splitting enzyme in the intestines of albino rats. Biochem. J. **31**: 560–564 (1937).
20. POSTERNAK, S. Sur la matière phospho-organique de réserve des plantes à chlorophylle. Procédé de preparation. Note de M. S. Posternak, presentée par M. Armand Gauteer. Compt. rend. **137**: 202–204 (1903).
21. SNOOK, L. C. Cereal grains as a source of nutritionally useful phosphorus. Empire J. Exptl. Agr. **6**: 20–24 (1938).
22. WILCOX, E. V., and KELLEY, W. P. Investigations on soils and fertilizers in Hawaii. Hawaii Sta. Rept. 16 (1914).

IMPORTANCE OF MALT EXTRACTION IN THE DETERMINATION OF AMYLASE ACTIVITIES [1]

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ABSTRACT

Two laboratory methods of extracting malt which will give the highest or maximum alpha-amylase values are described. The regular method involves extraction with 0.4% sodium bicarbonate solution at 30°C. for two hours; in the rapid method the extraction is conducted by stirring the malt with this solution at 35°C. in a Waring Blendor for 10 minutes. The maximum alpha-amylase values obtained by these methods are more representative of those experienced with malts in the brewing, distilling, and baking industries than the activities obtained with distilled water extracts. The temperature coefficient of dextrinization (Q_{10}, 30°/20°C.), was found to be 1.9 when the temperature effect on the color comparison is eliminated; as this coefficient is constant for different malts and extraction conditions, the alpha-dextrinizing activity can be determined either at 20°C. or 30°C. and the results calculated for both.

Although diastatic power determinations on distilled water extracts of barley malts do not reflect the activities realized commercially as well as extracts prepared by the regular method, they are sufficiently accurate for comparative purposes. Wheat malts may contain large quantities of salt-soluble beta-amylase, and hence reducing-sugar determinations on distilled water extracts fail to reveal their potential saccharifying activity when used in the distilling and baking industries.

In a recent study the authors discussed how the extraction of barley and wheat malts with dilute salt solutions results in extracts with markedly higher alpha-amylase activity and also higher total saccharifying power than extracts made with distilled water (5). The most suitable conditions for obtaining extracts of the highest amylase activity designated as maximum alpha-amylase activity were extraction with 0.05 to 0.2 *N* solutions of sodium acetate, bicarbonate, nitrate, or secondary phosphate at 30°C. Employing these conditions, the maximum alpha-amylase values of the extracts were relatively insensitive to variations in salt concentration within the foregoing concentrations, to extraction temperatures between 30° and 45°C., and to increasing the extraction time beyond the minimum necessary for complete dispersion.

In the present paper, two laboratory methods are described for the routine determination of maximum alpha-amylase activity in malt.

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Methods for Routine Determination of Maximum Alpha-Amylase Activity

In principle, the determination of maximum alpha-amylase activity involves the application of modified extraction procedures to the dextrinization methods of Sandstedt, Kneen, and Blish (14) and Olson, Evans, and Dickson (9, 10).

Choice of Salt. Sodium bicarbonate was selected as an appropriate solvent since 0.2 to 0.4% solutions will give maximum and consistent alpha-amylase values while 0.5 to 2.0% solutions of sodium acetate, biphosphate, and nitrate are required to obtain equivalent but less consistent activities.

Temperature and Duration of Extraction. The extraction may be conducted at any temperature between 30° and 45°C. provided the extraction time is adequate. The time necessary to secure maximum extraction is shortened when the temperature is raised (or the infusion is agitated) (5). This is illustrated in Fig. 1 in which the percentages of maximum alpha-amylase activity of a typical brewers' barley malt are plotted against time of extraction with 0.4% sodium bicarbonate solution at 20°, 30°, and 40°C. respectively. Maximum activities were obtained in about 1 hour at 30°C., and in 30 to 40 minutes at 40°C.

When the malt infusion was agitated in a Waring Blendor, maxi-

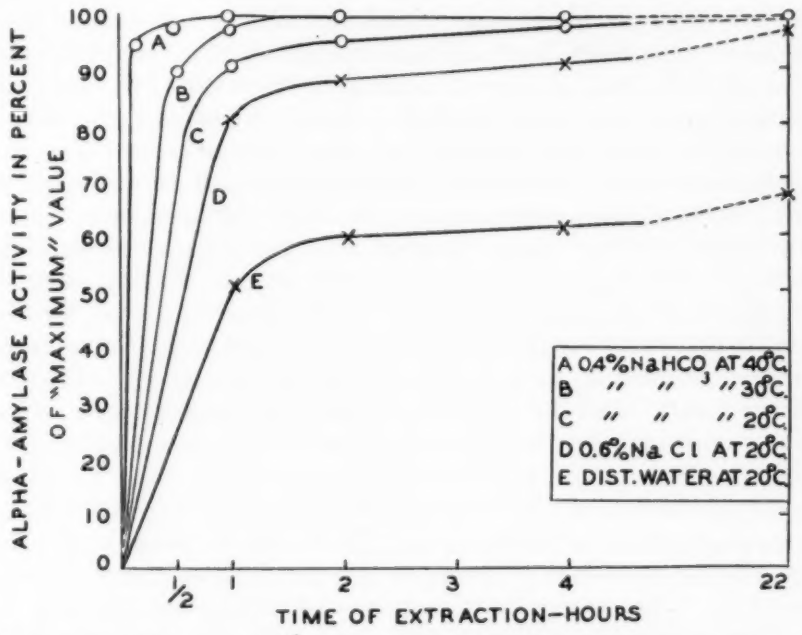

Fig. 1. Effect of time of extraction on alpha-amylase activity in the extract of an average brewers' barley malt. Comparison of extractions with 0.4% $NaHCO_3$ solutions at 20°, 30°, and 40°C., 0.6% NaCl solution at 20°C., and distilled water at 20°C.

mum values were obtained in 10 minutes at 35° to 40°C. Little or no destruction of alpha-amylase occurs if the pH is maintained between 5.0 to 8.0.

Dextrinization. Olson, Lowry, and Dickson (11) failed to obtain a constant relation for different malts between the alpha-amylase activities of distilled water extracts at 20° and 30°C. and determined at 20° and 30°C. respectively. The variability in the ratio between the activities for these temperatures was not reduced by extracting with 0.5% sodium chloride solution, although the average ratio was lower. The lack of a constant relation between the activities obtained at 30° and 20°C. was not due to the dextrinization step of the test since dextrinizations conducted at 20° and 30°C., for a series of malts extracted with distilled water at 20°C., gave a temperature coefficient (Q_{10}, 30°/20°) for dextrinization of 2.13 which was relatively constant for the different malts. In this study the dextrin color comparisons were made at the temperatures at which the dextrinizations were carried out so that the temperature coefficient includes any effect of the 10° difference in temperature on the dextrin-iodine color comparison.

Similar studies were conducted by the authors in which seven malts of varying activity were extracted with distilled water and with a variety of different salt solutions (0.5 to 2.0 *N*) at temperatures of 20°, 30°, and 40°C. for different periods of time. Dextrinization measurements were conducted with each extract at 20°C. and 30°C., but the color comparisons were all made at 20°C. The temperature coefficients in all these trials were within ±4% of 1.88; as the maximum deviation was well within the limits of experimental error, the coefficient is considered to be constant and independent of the nature of the malt, the extraction medium, or of the time and temperature of the extraction. To determine whether the differences between this coefficient and that of 2.13 found by Olson *et al.* could be ascribed to the effect of temperature on color comparisons noted by Redfern (12), samples of 10 malts were extracted with different solutions for varying times and temperatures. The dextrinizations were conducted at 30°C., but the color comparisons were made at both 30° and 20°C. Alpha-amylase activities calculated from the end point at 30°C. divided by those at 20°C. yielded a mean factor of 1.15. Multiplying the coefficient 1.88 by this factor gives a value of 2.16 which is in good agreement with the Q_{10} of 2.13 found by Olson *et al.* The temperature coefficient of the dextrinization reaction proper is therefore 1.88. The temperature coefficient of saccharification from the data for maltose formation at 30°C. and 20°C. has also been determined under conditions paralleling those reported for dextrinization.

It was found to fall between 1.80 to 1.84, so that the temperature coefficients for both phases of starch hydrolysis are of the same order of magnitude.

Methods. The studies which have been described provide a basis for the establishment of convenient routine methods for the determination of maximum alpha-amylase activity of malts. The use of suitable salt solutions eliminates the influence of temperature on the quantity of alpha-amylase extracted. Since the dextrinization values at 20° and 30°C. bear a constant relation to each other, the test can be conducted at either temperature according to convenience. Two methods, a regular and a rapid method, are described.

REGULAR METHOD: Twenty-five grams of finely ground malt are extracted with 500 ml. of 0.4% sodium bicarbonate solution at 30°C. for 2 hours and filtered at room temperature. Ten milliliters of the clear filtrate are diluted to 100 ml. with distilled water, adjusted to 20°C., and the dextrinization time determined on a 10-ml. aliquot as described by Olson, Evans, and Dickson (9, 10). The color comparison may be made in accordance with this method or with the glass end point color standard of Redfern (12).

RAPID METHOD: Five grams of ground dry malt, or 10 g. of whole wet malt are added to the glass or aluminum bowl of a Waring Blendor containing 500 ml. of 0.4% sodium bicarbonate solution which has been brought to about 35°C. The mixer is operated for 10 minutes at low speed for ground malt, and at high speed for whole malt. The malt infusion is then filtered through a fluted filter sufficiently large to take the entire change, the first 100 to 200 ml. being returned to the filter. A portion of the clear filtrate is brought to 20°C. and the dextrinization time determined on an aliquot as described by Olson, Evans, and Dickson (9, 10). With dry malts of moderate activity the determination is made with a 10-ml. aliquot (0.1 g. malt); with highly active malts a 5-ml. aliquot is taken. For malts of exceptionally low activity, double the usual quantities are extracted. The moisture content of wet malts is determined at the time the samples are weighed for extraction.

This method does not require a water bath for the extraction and the entire determination can be completed in less than an hour. Comparison of the values with those obtained by the regular methods is shown in Table I. For dry malts the results are in satisfactory agreement,[4] but somewhat greater differences were obtained between the values for fresh malts analyzed by the rapid method and those for the corresponding dried malts by the regular method; this appears to be

[4] Similar agreement of results was found when the two methods were compared using suitable salts other than sodium bicarbonate.

TABLE I

COMPARISON OF ALPHA-AMYLASE VALUES OBTAINED BY REGULAR AND RAPID METHODS

Sample number	Alpha-amylase activity (20°C. dextrinizing units)	
	Regular method	Rapid method
Brewers' barley malt		
1	33	33
2	33	33
3	33	33
4	29	28
5	31	32
6	29	28
7	31	32
Distillers' barley malt		
8	44	44
9	40	40
10	30	30
Malted wheat flour		
11	18	18
Green barley malt		
12	35[1]	40[2]
13	47[3]	49[2]

[1] Vacuum dried.
[2] Wet.
[3] Kiln dried.

due to changes in activity occurring during dehydration rather than to the methods.

Determination of Saccharifying Activity; Diastatic Power

The diastatic power of malt reflects the composite saccharifying activities of beta- and alpha-amylase and perhaps other enzymes. When Kjeldahl in 1879 (6) and Lintner in 1885 and 1908 (7, 8) introduced this assay, they infused the malt at room temperature for 6 hours and assumed that the extraction of diastase was complete. The lack of agreement in the results obtained in different laboratories led to extensive collaborative work to develop an acceptable standardized procedure (1, 2, 3). Variations in the conditions of extraction were found to be one source of error (13). Strict adherence to the prescribed temperature, time, and concentration is necessary to assure reproducible results, especially since the conventional conditions (malt ratio, 1:20; 20°C.; and 2.0 or sometimes 2.5 hours) do not give complete extraction of the amylases. The authors have shown that the extraction of alpha-amylase from both barley and wheat malt is markedly influenced by salts and by temperature and this must in turn affect the diastatic power. The extraction of beta-amylase from barley malt is little affected by these variations in the conditions of extraction, but this does not appear to be true for wheat malts. It is

therefore necessary to consider separately the most appropriate conditions for determining the diastatic power of barley and wheat malt.

Barley Malt. The extraction conditions shown to give maximum alpha-amylase activities are approximately those used in technical mashing operations. The salts most suitable for this purpose slightly stimulate beta-amylase at 20° and 30°C. in solution, but apparently are not important in its release (5). Extraction at 40°C. and for 1 hour results in some destructive heat effect which about compensates that stimulation. It may be assumed that conventional malt extraction at 20°C. with distilled water yields almost the same amount of beta-amylase activity as is obtained in industrial operations, and that the actual increase in diastatic power is practically that of the increased alpha-saccharifying activity. Table II illustrates these effects on commercial barley malts.[5]

The increase in alpha-amylase activity due to the use of salts and higher extraction temperatures raises the diastatic power above that determined in the conventional manner by extracting with distilled water at 20°C. The two values for this increase under sections A and B of the table represent the range of variation which may occur with brewers' and distillers' malts under practical conditions; the smaller figure is for malts of high diastatic power with a relatively small increase in alpha-amylase activity; while the larger figure applies to the reverse condition. Although the ratio of the alpha-amylase activities for salt extracts as compared with water extracts varies considerably, the variations in the effect upon diastatic power are well within the commonly accepted margin of experimental error. The conventional diastatic power determination, therefore, can serve for the comparative evaluations of malts of the same general type. If values more strictly conforming to technical mashing conditions are desired, the extraction procedure described as the regular method may be employed, or the extraction can be made with industrial water at 40°C. for 1 hour. The rapid method is not suitable for this purpose because the vigorous stirring leads to a decrease in beta-amylase activity.

Wheat Malt. The influence of extraction conditions on the diastatic power of the wheat malts shown in Table II is strikingly different from that found with barley malts because of the presence of large quantities of salt-soluble beta-amylase in the former. The increase in beta-amylase activity due to extraction with salts and to increasing the temperature or duration of extraction so markedly influenced the diastatic power that it greatly overshadowed the higher alpha-saccharifying activity.

[5] Beta-amylase activity was calculated as the difference between the maltose equivalents of the total saccharifying power and of the alpha-dextrinizing activity. We used the mean values of these equivalents as published by Olson, Evans, and Dickson (9) and by Ehrnst and Lucht (4) which are somewhat divergent at higher alpha dextrinizing values.

TABLE II

INFLUENCE OF SALT AND TEMPERATURE DURING EXTRACTION ON DIASTATIC POWER AND BETA-AMYLASE

Extracting medium	Diastatic power in % of conventional maltose units			Effect upon D.P. of alpha-amylase increase in % of conventional D.P.			Beta-amylase in % of distilled water-values at 20°C.		
Temp. of extraction Hours of extraction	20° 2	30° 2	40° 1	20° 2	30° 2	40° 1	20° 2	30° 2	40° 1
A. COMMERCIAL BREWERS' BARLEY MALTS[1] (100°–125° L.; 30–35 Maximum alpha, 20° Dextrinizing Units)									
Distilled water	100	101	102	0	3–4	4–5	100	98	96
0.6% NaCl	108	108	108	7–8	7–8	7–8	101	101	101
0.4% $NaHCO_3$	111	116	110	7–10	7–10	7–10	104	108	100
B. COMMERCIAL DISTILLERS' BARLEY MALTS[2] (180°–200° L.; 40–45 Maximum alpha, 20° Dextrinizing Units)									
Distilled water	100	104	103	0	2–3	3–4	100	102	99
0.6% NaCl	104	108	105	4–6	5–7	6–8	100	103	99
0.4% $NaHCO_3$	105	108	107	5–7	6–9	6–9	100	101	100
C. WHEAT MALT (106° L.; 33 Maximum alpha, 20° Dextrinizing Units)									
Distilled water	100	105	107	0	2	3	100	104	105
0.6% NaCl	146	155	164	4	4	3	155	167	167
0.4% $NaHCO_3$	147	153	149	3	4	3	158	160	160
D. COMMERCIAL MALTED WHEAT FLOUR (57° L.; 18 Maximum alpha, 20° Dextrinizing Units)									
Distilled water	100	123	137	0	5	3	100	122	139
0.6% NaCl	160	170	—	3	10	—	169	174	—
0.4% $NaHCO_3$	—	165	—	—	10	—	—	167	—

[1] The analytical values are the means for two brewers' malts, dried at final temperatures of about 72°C.

[2] The results are the means for three distillers' malts dried at about 50°C.

These limited results suggest that distilled water extracts of wheat malt are unsatisfactory for the reliable determination of amylase activity. Neither the conventional diastatic power test nor the alpha-amylase determination on such extracts may reflect the enzymic activities of wheat malt for use in baking or in the distilling industry. Further studies are necessary to develop a suitable extraction procedure.

Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (5th ed.). Am. Assoc. Cereal Chemists: St. Paul, Minn. (1947).
2. AMERICAN SOCIETY OF BREWING CHEMISTS. Methods of analysis (1st ed.) Am. Soc. Brewing Chemists (1935).
3. AMERICAN SOCIETY OF BREWING CHEMISTS (4th ed.). Am. Soc. Brewing Chemists (1944).

4. EHRNST, L. E., and LUCHT, O. C. A revised method for the determination of alpha-amylase and beta-amylase in malt. Am. Soc. Brewing Chemists Proc. pp. 19–29 (1947).
5. ERLICH, V. L., and BURKERT, G. M. Influence of salts on amylase activities of malt. Cereal Chem. **26**: 239–257 (1949).
6. KJELDAHL, M. J. Recherches sur les ferments producteurs de sucre. Compt. rend. trav. lab. Carlsberg **1**: 109–157 (1879). Also published in Z. ges. Brauw. **3**: 49 a.f. (1880).
7. LINTNER, C. J. Studien über Diastase. I. Z. ges. Brauw. **8**: 281–285 (1885). Also published in J. prakt. Chem. neue Folge. **34**: 378–394 (1886).
8. LINTNER, C. J., and WIRTH, C. Ueber die Bestimmung der diastatischen Kraft des Malzes und von Malzextrakten. Z. ges. Brauw. **31**: 421–425 (1908).
9. OLSON, W. J., EVANS, RUTH, and DICKSON, A. D. A modification of the Kneen and Sandstedt methods for the determination of alpha- and beta-amylases in barley malt. Cereal Chem. **21**: 533–539 (1944).
10. OLSON, W. J., EVANS, RUTH, and DICKSON, A. D. A corrected unit of alpha-amylase activity. Cereal Chem. **24**: 299–300 (1947).
11. OLSON, W. J., LOWRY, M. T., and DICKSON, A. D. The effect of sodium chloride in the extraction of malt at 20° and 30°C. on alpha-amylase and diastatic power values. Am. Soc. Brewing Chemists Proc. pp. 13–19 (1948).
12. REDFERN, S. Methods for determination of alpha-amylase. IV. A glass end point color standard for use in the dextrinizing method; effect of temperature and starch lot on this method. Cereal Chem. **24**: 259–268 (1947).
13. SALLANS, H. R., and ANDERSON, J. A. Sources of error in the determination of the diastatic power of malt. Cereal Chem. **14**: 708–720 (1937).
14. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16**: 712–723 (1939).

BREEDING FOR NIACIN CONTENT IN A SORGHUM CROSS, WESTLAND X CODY [1]

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ABSTRACT

The niacin content of grain from F_1, F_2, and F_3 plants of a Westland × Cody cross is presented.

Westland ranged from 43.0 to 49.1 μg. niacin per gram and Cody ranged from 66.9 to 72.9 μg. per gram. The grain from the F_1 plant contained 46.3 μg. per gram. The grain from the F_2 plants contained niacin in concentrations ranging from 37.8 to 103.6 μg. per gram. Grain from one F_3 plant had a niacin content of 124 μg. per gram, the highest value thus far found for sorghum.

Niacin content appears to be an inherent varietal characteristic. The development of sorghum varieties with high niacin content appears to be possible.

The need for dietary niacin by certain classes of animals including swine, poultry, and human beings has been well established, although

[1] Manuscript received January 14, 1949.
Contribution from the Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, Peoria, Illinois; the Fort Hays Branch Experiment Station, Hays, Kansas, Kansas State Agricultural Experiment Station, Manhattan, Kansas, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture. Contribution No. 48 Department of Fort Hays Branch Station.
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the physiology and biochemistry of this vitamin remain obscure. Recent tests from numerous laboratories have indicated that the amount of dietary niacin required may depend upon the quality of the protein in the diet. Increased niacin requirements have been noted with rations compounded with certain proteins, especially those deficient in tryptophane. In view of the general deficiency of this amino acid in the proteins found in cereal grains and of the biological interrelationship between niacin and tryptophane, the niacin content of grains apparently becomes an important characteristic to be considered in formulating rations.

Little is known concerning factors influencing the vitamin content of plant materials. Several factors undoubtedly play important roles, of which climatic conditions, soil types, and fertilizer as affecting nutrition of the plant have been studied in some instances. The literature pertaining to the influence of some of these factors has been reviewed by Somers and Beeson (8). In general, however, the inheritance of vitamin content has received little attention.

A few reports are available which suggest that perhaps the content of B-complex vitamins in cereals may be altered by breeding new varieties. Burkholder, McVeigh, and Moyer (2) presented niacin analyses of 46 strains of corn which averaged 34.6 μg., but ranged from 18.2 to 62.1 μg. of niacin per gram on an air-dry basis. Sweetcorn varieties generally contained more niacin than dent varieties, and popcorn varieties averaged lowest in niacin. Barton-Wright (1) found that sweetcorn contained nearly twice as much niacin as the flint corns he analyzed (31.0 and 15.6 μg. per gram, respectively). Later, Mather and Barton-Wright (5) analyzed sugary and starchy kernels from the same open-pollinated ears. Presumably the starchy kernels were developed following pollination with starchy types and the sugary kernels following pollination by sugary types. In five such sets of samples analyzed, the sugary grain averaged 26.2 μg. and the starchy grain 14.0 μg. niacin per gram, with small individual differences attributable to strain characteristics. Richey and Dawson (6) concluded that corn hybrids with niacin concentrations as high as 50 μg. per gram probably could be developed. They suggested that two modes of niacin inheritance may function in corn. One involves the joint action of many genes of small individual effects with dominance lacking, and the second mode, following the suggestion of Mather and Barton-Wright (5), may involve Su su alleles, with Su dominant for lower niacin concentration.

Hunt, Ditzler, and Bethke (3) reported the niacin and pantothenic acid content of nine double-cross corn hybrids grown at five experiment stations in three successive years. Variations were found for

all crop years, and all locations, but the greatest variation in niacin content was ascribed to varietal influences. In Cody sorghum, on the other hand, Tanner, Pfeiffer, and Curtis (9) found that the niacin content in grain grown at Hays, Kansas, from 1942 through 1945 were 72.1, 72.9, 70.5, and 71.8 μg. per gram respectively. This would indicate high stability in the niacin level from year to year even though the seasons were widely different. Similar stability was obtained for several other sorghum varieties grown for a shorter period.

The data available on grain sorghums suggest that niacin content is a varietal characteristic that may be altered by breeding practices. Knox *et al.* (4) observed wide differences in the niacin content of 29 grain sorghum varieties grown under comparable conditions. Similar data were obtained by Tanner, Pfeiffer, and Curtis (9) for 48 commercial and experimental varieties from different sources and locations. The niacin content ranged from 27.7 to 91.9 μg. per gram on a moisture-free basis. Some strains developed by hybridization carried niacin levels much higher than either of the parental varieties. For instance, four Cody × Wonder Club strains grown at Hays, Kansas, in 1945 contained 76.6 to 91.9 μg. of niacin per gram; three of these were significantly richer in niacin than Cody, the high-niacin parent variety.

Since the data suggested that new lines of grain sorghum could be developed that would be rich in this vitamin, its inheritance in one series of samples, representing a cross of Westland × Cody, was studied. Westland is a commercial variety relatively low in niacin and Cody is a commercial variety relatively rich in niacin.

Methods and Materials

Niacin determinations were made by the microbiological method of Snell and Wright (7) after hydrolysis of samples in 1 *N* sulfuric acid for 30 minutes at 120°C. Hydrolyzates were adjusted to pH 6.8, filtered, and assayed in the conventional manner. All results are expressed on a moisture-free basis.

The seed samples were from individual F_2 plants of a Westland × Cody cross grown at Hays, Kansas, in 1945. After these strains had been analyzed, remnant seed was planted the following year, and random samples of the seed from F_3 plants were analyzed to check the stability of these new lines. Care was taken to include F_3 plants from parental lines ranging from low to high in niacin content.

Results

Seed from the specific plants of Westland and Cody used in this cross was not available, but pure lines of both varieties were analyzed

for two and five years, respectively. In Westland the niacin content ranged from 43.0 to 49.1 μg. per gram; for Cody the range was from 66.9 to 72.9 μg. per gram with only one sample, that of the 1946 crop, having less than 70 μg. per gram. The amount of niacin in Westland appeared to be stabilized at a relatively low level compared to Cody. The F_1 generation of the cross, grown at the Hays station in 1944 and assayed in 1947, contained 46.3 μg. of niacin per gram.

The F_2 generation plants were grown in 1945. Seed from 335 plants was harvested and analyzed. The niacin content ranged from a low of 37.8 to a high of 103.6 μg. per gram. Two samples of Cody produced the same crop year averaged 69.4 μg. per gram. The data for the 335 plants were grouped into classes each with a spread of 5 μg. per gram. Only seven plants had niacin content as low as or lower than the Westland variety, the low parent, while 127 plants contained niacin concentrations equal to or greater than Cody, the high-niacin parent, and 201 plants were intermediate between the parental varieties. In this group, the majority of plants were above the midpoint between the parents. This distribution of samples is shown graphically in Fig. 1.

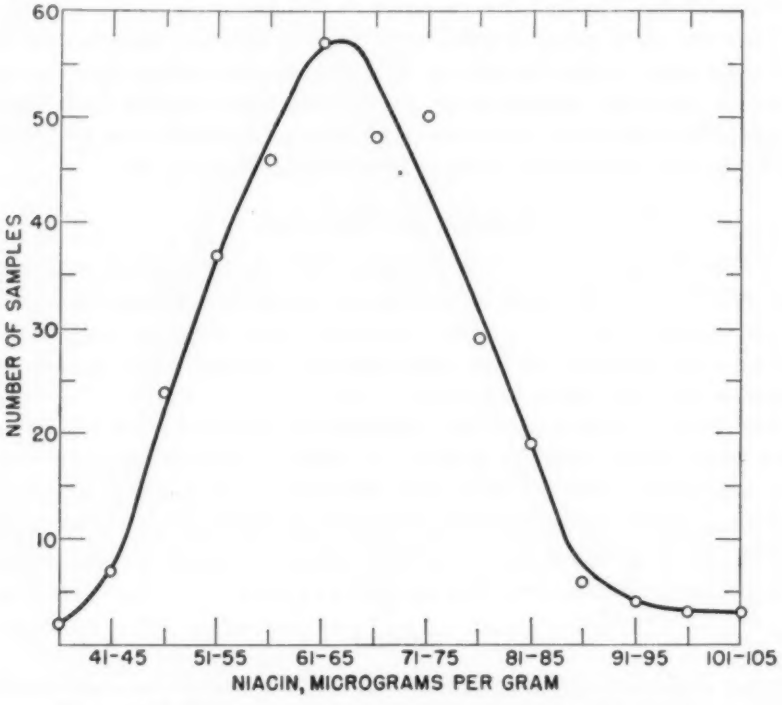

Fig. 1. Distribution of niacin in seed from F_2 plants of Westland × Cody cross.

No attempt was made to assay all F_3 heads for niacin because of the large number of samples involved. However, five groups were analyzed involving bulk seed and two or three plants from rows selected to include those produced from randomly selected F_2 plants of high, intermediate, and low niacin content. The niacin analyses of this group of samples are shown in Table I.

TABLE I

NIACIN CONTENT OF BULK SEED AND SEED FROM SEPARATE PLANT SELECTIONS OF F_3 GENERATION WESTLAND × CODY

Niacin content in	Groups				
	1	2	3	4	5
			μg./g.		
F_2 plant, 1945	102.3	63.1	46.9	43.3	39.3
F_3 plant, 1946 Plant 1	91.4	48.9	42.2	53.3	92.2
Plant 2	42.0	50.1	48.2	56.5	95.1
Plant 3	75.0	60.2	—	—	57.3
Bulk seed	84.4	51.3	54.7	79.3	45.3

In general the bulk seed produced on high-niacin rows tended to have a high-niacin concentration and that on low-niacin rows tended to be low. Segregation, however, occurred in each group. In Series 4, the bulk seed from the F_3 plants was considerably richer in niacin than the seed from the F_2 plants.

These data suggested that further analyses of the seed from F_3 plants would be necessary to indicate the potential range of variation in niacin content that might be expected. Additional analyses were made of 157 F_3 heads grown on 55 head-rows. Table II summarizes the results obtained. In only one group did the grain in F_3 heads

TABLE II

RANGE OF NIACIN IN SEED FROM F_2 AND F_3 PLANTS FROM THE CROSS WESTLAND × CODY, HAYS, KANSAS, 1945 AND 1946

No. of F_2 plants from 1945 propagated to F_3 in 1946	Range of niacin in the F_2 plants	Average niacin content F_2 plants, 1945	No. of F_3 plants analyzed, 1946	Range of niacin content of F_3 plants, 1946	Avg. niacin content F_3 plants, 1946
	μg./g.	μg./g.		μg./g.	μg./g.
5	38.0– 49.1	45.0	14	43.9– 74.5	59.4
19	50.9– 59.3	55.2	55	35.5–109.9	64.8
15	60.3– 69.8	63.8	43	53.7–115.9	75.8
7	70.8– 79.9	73.5	18	51.4–124.0	76.3
6	80.1– 81.6	80.7	18	69.9–106.3	92.2
3	90.6–102.0	94.6	9	68.0–105.6	88.2
55			157		

have an average niacin content below that of the F_2 heads. In one F_3 row a head containing 124.0 μg. of niacin per gram was found, which is the highest niacin concentration yet found in this cereal. This is more than 50 μg. per gram higher than the Cody variety, the initial high-niacin parent.

So far as could be determined, niacin content of sorghums was not associated with any observable plant character that could be used as a genetic tester. As is the case with many other quantitative characters, the inheritance of niacin probably is complex and results from the interaction of many factors.

While an analysis of only three heads per row is not sufficient to measure the behavior of F_3 rows, the data indicate that new lines of grain sorghum rich in niacin may be obtained. Other agronomic requirements also must be met, but if future research demonstrates the desirability of developing cereal varieties of greater nutritional value, it should be possible to increase the niacin content of grain sorghums materially by selection from suitable crosses.

Literature Cited

1. Barton-Wright, E. C. The microbiological assay of nicotinic acid in cereals and other products. Biochem. J. **38**: 314–319 (1944).
2. Burkholder, P. R., McVeigh, I., and Moyer, Dorothy. Niacin in maize. Yale J. Biol. and Med. **16**: 659–663 (1944).
3. Hunt, C. H., Ditzler, Lorraine, and Bethke, R. M. Niacin and pantothenic acid content of corn hybrids. Cereal Chem. **24**: 355–363 (1947).
4. Knox, Grace, Heller, V. G., and Sieglinger, J. B. Riboflavin, niacin, and pantothenic acid contents of grain sorghums. Food Research **9**: 89–91 (1944).
5. Mather, K., and Barton-Wright, E. C. Nicotinic acid in sugary and starchy maize. Nature **157**: 109–110 (1946).
6. Richey, F. D., and Dawson, R. F. A survey of the possibilities and methods of breeding high-niacin corn (maize). Plant Physiol. **23**: 238–254 (1948).
7. Snell, E. E., and Wright, L. D. A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139**: 675–686 (1941).
8. Somers, G. F., and Beeson, K. C. The influence of climate and fertilizer practices upon the vitamin and mineral content of vegetables. Advances in Food Research **1**: 291–324 (1945).
9. Tanner, F. W., Pfeiffer, Shirley E., and Curtis, J. J. B-complex vitamins in grain sorghums. Cereal Chem. **24**: 268–274 (1947).

DETERMINATION OF REDUCED IRON IN VITAMIN ENRICHMENT MIXTURES [1]

J. D. NEUSS and H. A. FREDIANI [2]

ABSTRACT

When iron is present as reduced iron (iron by hydrogen) in vitamin enrichment mixtures it may be determined readily by magnetic separation and direct weighing. Such a method provides a rapid and precise method for determining the added iron content and precludes the inclusion of other iron compounds that may be present in the other components of the mixture.

The official A.O.A.C. method (2) for determining iron in cereal foods, including bread, involves wet ashing of the sample, reduction to the ferrous state with hydroxylamine hydrochloride, subsequent color development with alpha-alpha dipyridyl or orthophenanthroline, and measurement in a suitable colorimeter using applicable calibration curves. In somewhat similar fashion the official A.A.C.C. method (1) for iron in flour involves ashing of sample, dissolution of residue, reduction with hydroquinone, and colorimetric measurement with dipyridyl. No official method has been published for this determination in vitamin enrichment mixtures, so that current practice appears to be to utilize suitable modifications of the method used for flour. When the iron in such a mixture is present in the metallic state in the form of reduced iron, a magnetic flotation method may be utilized for the physical separation of the iron from the other nonmagnetic components. The sample is placed in a beaker supported on one pole of a strong Alnico magnet. The major portion of the vitamins and starch is removed by flotation with methanol or denatured ethanol. Several washings with distilled water then dissolve the remaining riboflavin not removed by the initial flotation. The residual iron is washed with methanol to remove water and finally rinsed with ethyl ether to remove methanol. Air drying for several minutes suffices to yield the iron in weighable form.

Materials and Methods

Into a tared 100-ml. beaker accurately weigh 1.0000 g. of the well-mixed sample of the vitamin mixture. Place this beaker on one pole of a large Alnico magnet, similar to catalog No. 12–012, Fisher Scientific

[1] Manuscript received April 26, 1949.
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Co. Add 15-25 ml. of reagent grade methanol, simultaneously washing down the inside wall of the beaker. Agitate the sample vigorously for 15–30 seconds with a stirring rod bearing a rubber policeman. Keep the base of the beaker against the pole of the magnet in the following and all subsequent washings and decantations. Rinse and remove the stirring rod. Decant the methanol suspension and repeat the flotation of nonmagnetic constituents with a second portion (15–25 ml.) of alcohol. Follow this with four successive washes using distilled water. The third water wash should be only faintly yellow and the final water wash should be colorless. Wash again with two portions of methanol and complete the washing process with two portions of ethyl ether. Let the beaker and residue of iron stand for 2–3 minutes until iron particles are dry. Upon removal of the beaker from the magnet the iron particles will be free-flowing and loose if properly washed and dried. Weigh the beaker with iron and note weight, in milligrams, of iron recovered. Multiply this value by 28.35 to obtain the iron content of the sample in milligrams per ounce.

TYPICAL DATA

Sample	A_1	A_2
Wt. sample	1.0000	1.0000
Wt. iron recovered	0.1764	0.1775
Average wt. iron recovered	177 mg.	
Mg. iron per ounce	5020	

Results and Discussion

Replicate samples have been found to be easily reproducible to 1–2 mg. (1%). In a series of five determinations carried out on a uniform sample the average deviation was found to be 0.5%, with a maximum deviation of 1%. The iron recovered from a series of 15 determinations was assayed by standard methods and found to be 97.5% pure, indicating negligible contamination by other components of the original mixture.

The adequacy of air drying of the iron residue was tested by further drying at 110°C. The additional loss was generally negligible and in no case exceeded 0.5 mg.

In Table I are listed the results obtained on 12 different samples both by the magneto-gravimetric and by a volumetric method. In this comparison, because of the relatively high iron contents of the samples studied, it was not found feasible to use the colorimetric A.O.A.C. procedure. Excessive dilution or unreasonably small samples would have been necessary in order to obtain reasonable color intensities. In the volumetric method used, the sample was ignited in a muffle, the residue then dissolved in hydrochloric acid, and reduced by passage through a Jones reductor. The ferrous iron

TABLE I

DETERMINATION OF REDUCED IRON IN VITAMIN ENRICHMENT MIXTURES

Sample	Magneto-gravimetric method mg. Fe per oz.	Volumetric method mg. Fe per oz.	Difference mg.
1	4977	4950	+27
2	4946	4890	+56
3	4976	5020	−44
4	4828	4970	−142
5	5105	5040	+65
6	4863	4890	−27
7	5093	5030	+63
8	4989	4850	+139
9	4852	4960	−108
10	4829	4750	+79
11	4977	4950	+27
12	4946	4890	+56

thus formed was titrated with standard ceric sulfate solution. The recommendations of Edmonds and Birnbaum (3) and of Smith (4) were generally followed in this part of the procedure. The average relative difference between the two methods was found to be 1.5%.

Acknowledgment

Acknowledgment is made to Mr. Walter Fulmer and the staff of the Vitamin Testing Section for the results obtained by the Ceric Sulfate method.

Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (5th ed.). Am. Assoc. Cereal Chemists: St. Paul, Minn. (1947).
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis (6th ed.). Assoc. Official Agr. Chemists: Washington, D. C. (1945).
3. EDMONDS, S. M., and BIRNBAUM, N. Microdetermination of iron with the silver reductor. Ind. Eng. Chem. (Anal. Ed.) **12**: 60–61 (1940).
4. SMITH, G. F. Cerate oxidimetry. G. Frederick Smith Chemical Co.: Columbus, Ohio (1942).

BOOK REVIEWS

Seed Crushing, Compound and Provender Milling. By H. Moore and A. S. Moore. Volume II. 238 pp. Northern Publishing Co., Liverpool, England. Price: 10/-.

The present work is an extension of Volume I, with much greater emphasis on the subjects which originally were treated only briefly, or not at all. The series has been prepared for use as texts in courses offered by the Seed Crushing, Compound and Provender Manufacturing Industries. Both books are divided into two sections, Part I, "Seed Crushing," and Part II, "Compound and Provender Milling." In both cases, the technology of various operations conducted in oil and feed mills is described in excellent detail, with drawings illustrating clearly the functioning of the various machines employed in all steps of manufacturing. The methods and equipment, however, are entirely English and in most cases are at least slightly

different from those known to oil and feed producers in the United States. In many instances, the design of equipment is vastly different from that of American counterparts, but the mechanical features of the English machines deserve careful consideration because of rather obvious advantages over some of the equipment used in this country.

Under "Seed Crushing," Volume II contains much more information than does Volume I with regard to continuous screw presses, solvent extraction, and the handling of oil and meal. Even so, the types of screw presses and solvent extractors used in this country are not described. To do so, and to describe in equal detail the many other types of such equipment used throughout the world, would necessitate tremendous expansion of the book into something more than a text prepared for a specific course of instruction.

The second volume differs from the first with respect to the descriptions of compound and provender milling also. In English terminology, "compound" is the equivalent of mixed feed in the United States, whereas "provender" refers to such items as mill feed, hominy feed, and the like. The new volume is particularly complete in its descriptions of conveyors and equipment for handling solid materials. Among the other subjects treated quite fully, and which were not covered in the earlier book, are grain storage, drying, and size reduction.

Volume I and Volume II of this series are complementary, i.e., Volume II is not simply a revision of Volume I, but a presentation of information that could not be included in the course for which Volume I was prepared as a text. Both books should prove of great value to anyone interested in the actual operating details of an oil mill or feed plant, even though much of the English equipment described might be quite unfamiliar to him. Equivalent texts describing American machinery and methods would be of enormous value to those on the operating level in American mills.

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Theory of the Stability of Lyophobic Colloids: The Interaction of Sol Particles Having an Electrical Double Layer. By E. J. W. Verwey and J. Th. G. Overbeek. Natuurkundig Laboratarium N. V. Philips Gloeilampenfabrieken, Eindhoven (Netherlands). XI + 205 pp. 54 figs. 16.5 × 24 cm. With the collaboration of K. Van Nes Elsevier Publishing Company, Inc., 215 Fourth Avenue, New York 3, N. Y. 1948. Price $4.50.

The purpose of this book is to develop a quantitative theory of the stability of hydrophobic colloids and suspensions in terms of the present day concepts of the electrochemical double layer and of the London–van der Waals forces. The basic idea involved in this theory is that a repulsion force, in combination with an attraction force, determines the repulsion or attraction potential which will function between the particles of disperse phase and defines the conditions of stability, or lack of stability, which will exist. The repulsion force results from the mutual interaction of the diffuse double layers, while the attraction force is due to London–van der Waals forces.

Roughly a third of the book is devoted to a treatment of the modern concepts of the structure of the electrical double layer and the nature of van der Waals forces. This discussion serves to orient the reader for the ensuing treatment of the theory of stability which is developed. The remainder of the book treats this theory from the standpoints of interactions which will occur between two parallel flat plates, as one case, and between spherical colloidal particles, as a second case. Predictions based upon the theory are shown to agree well with many of the experimental observations of colloid chemistry. For example, a theoretical basis for the Schultz-Hardy effect of electrolytes upon the stability of lyophobic colloids is given which shows good agreement with experiment. The theoretical work on the stability of lyophobic systems by previous authors is discussed in an appendix to this book.

The authors have performed a great service to colloid chemistry in their logical development of a theory in explanation of this very complex phenomenon. While further elaboration of the theory involved will undoubtedly be required, the present

treatment constitutes a remarkable and stimulating advance in the understanding of the factors involved in the stability of hydrophobic colloids and suspensions.

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The Chemistry and Technology of Enzymes. By Henry Tauber. 550 pp. John Wiley & Sons, Inc., New York and Chapman & Hall, Ltd., London. 1949. Price $7.50.

This book replaces two by the same author, namely *Enzyme Chemistry* (1937) and *Enzyme Technology* (1943) which have not gained wide usage as standard references for students. In general the structure of the earlier books has been retained but the contents have been rewritten and considerably enlarged.

In Part 1, 15 chapters are devoted to enzyme chemistry, and methods for the preparation of several enzymes are included. The initial definition and classification of enzymes is slightly confusing. For example, cytochrome is placed in the iron-porphyrin enzyme group together with the remark that "the cytochromes . . . are not enzymes."

In Part 2, 16 chapters are concerned with the methods used in the industrial production of enzymes and their uses, and with the production of industrially important organic compounds by fermentation. In the last chapter, microbiological methods for the estimation of vitamins and amino acids are discussed. Naturally, in any one book which aims at covering such a broad field as does this one, there is a limit to the amount of discussion on the various topics. However, the author has covered the important aspects of his subjects. While most cereal chemists will probably disagree with the author in his statements on the role of proteinases in breadmaking, they will find that this book contains much useful information and many references on matters of interest to them.

The chemical formulae, graphs, and photographs are very well presented and the occasional errors, such as "Streptococcus aureus" instead of "Staphylococcus aureus," are relatively unimportant.

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General. Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, all original drawings or photographs for figures, and one set of small photographic reproductions of figures.

Issues of *Cereal Chemistry* published after January 1, 1948, when some changes were made, are the most useful guides to acceptable arrangements and styling of papers.

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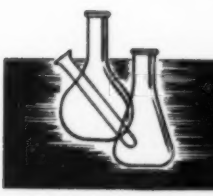

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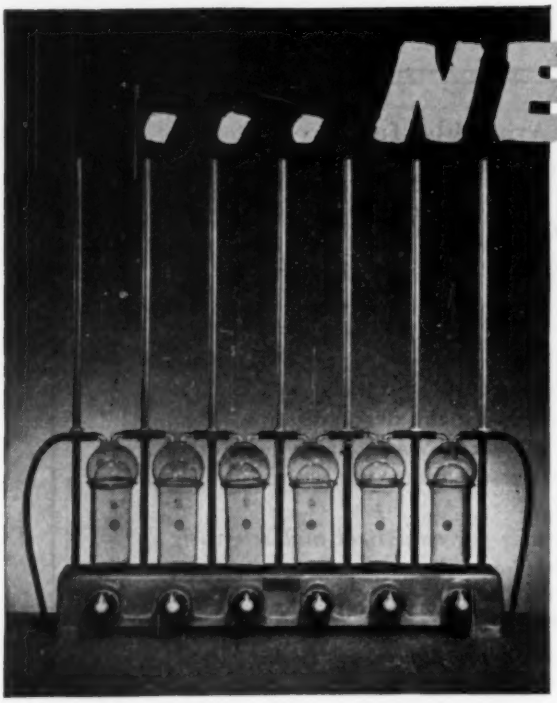

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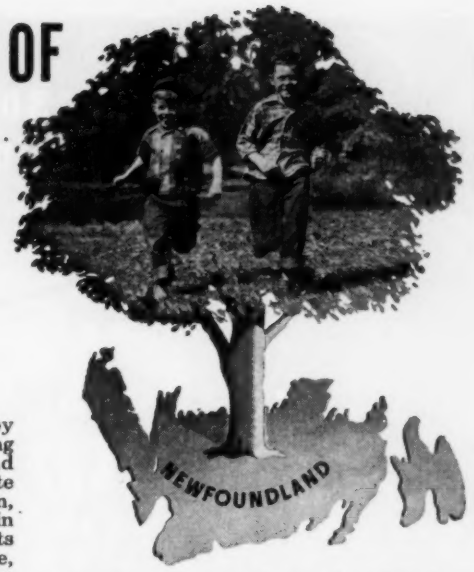

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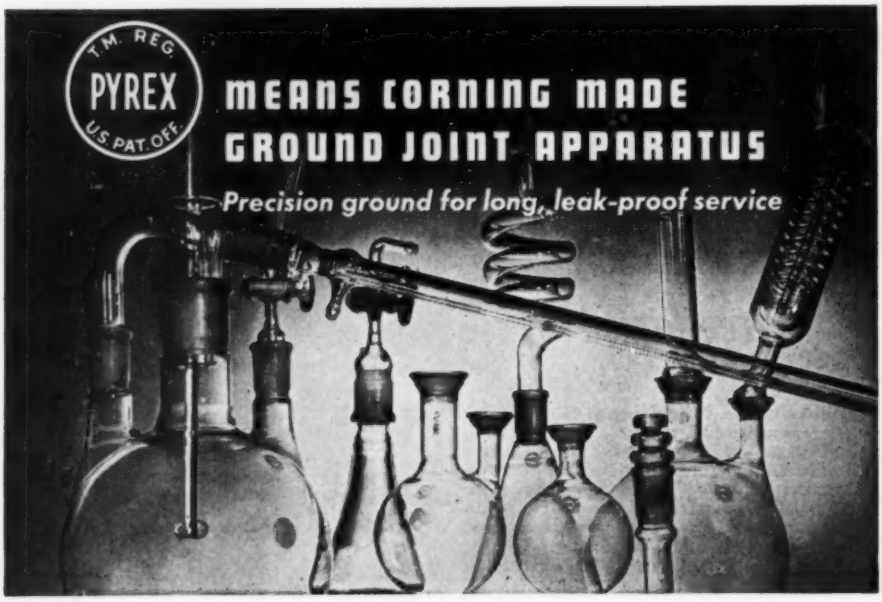

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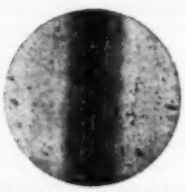

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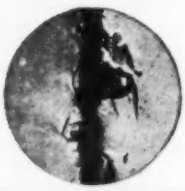

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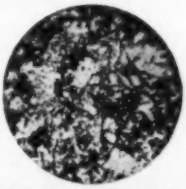

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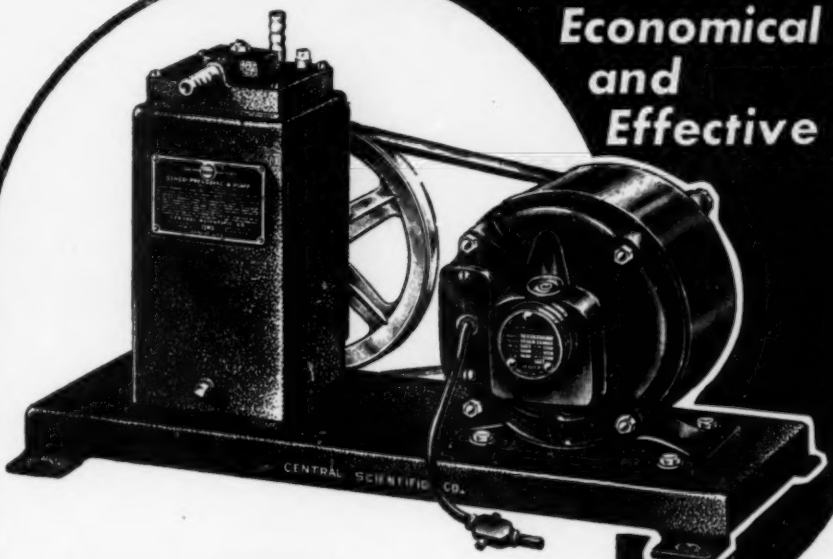

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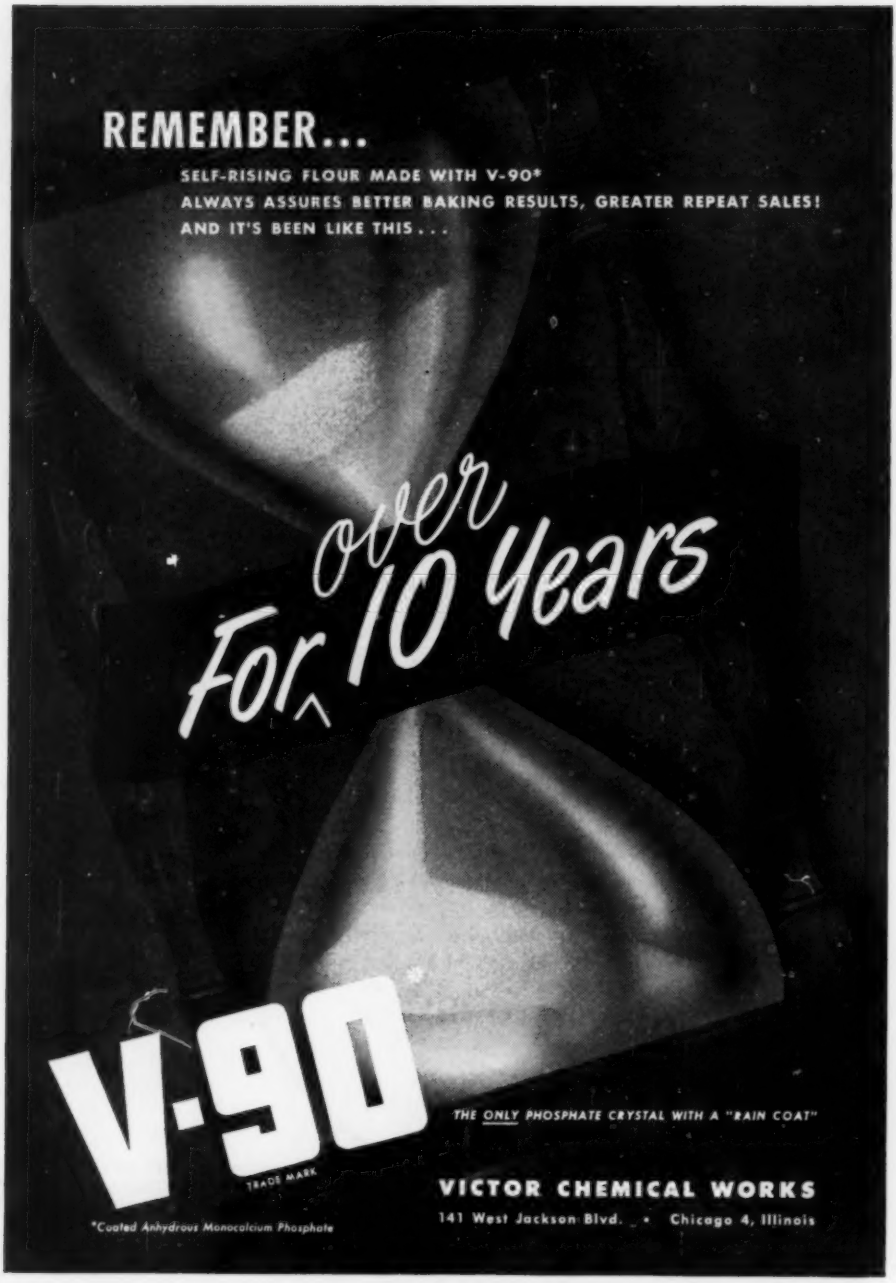
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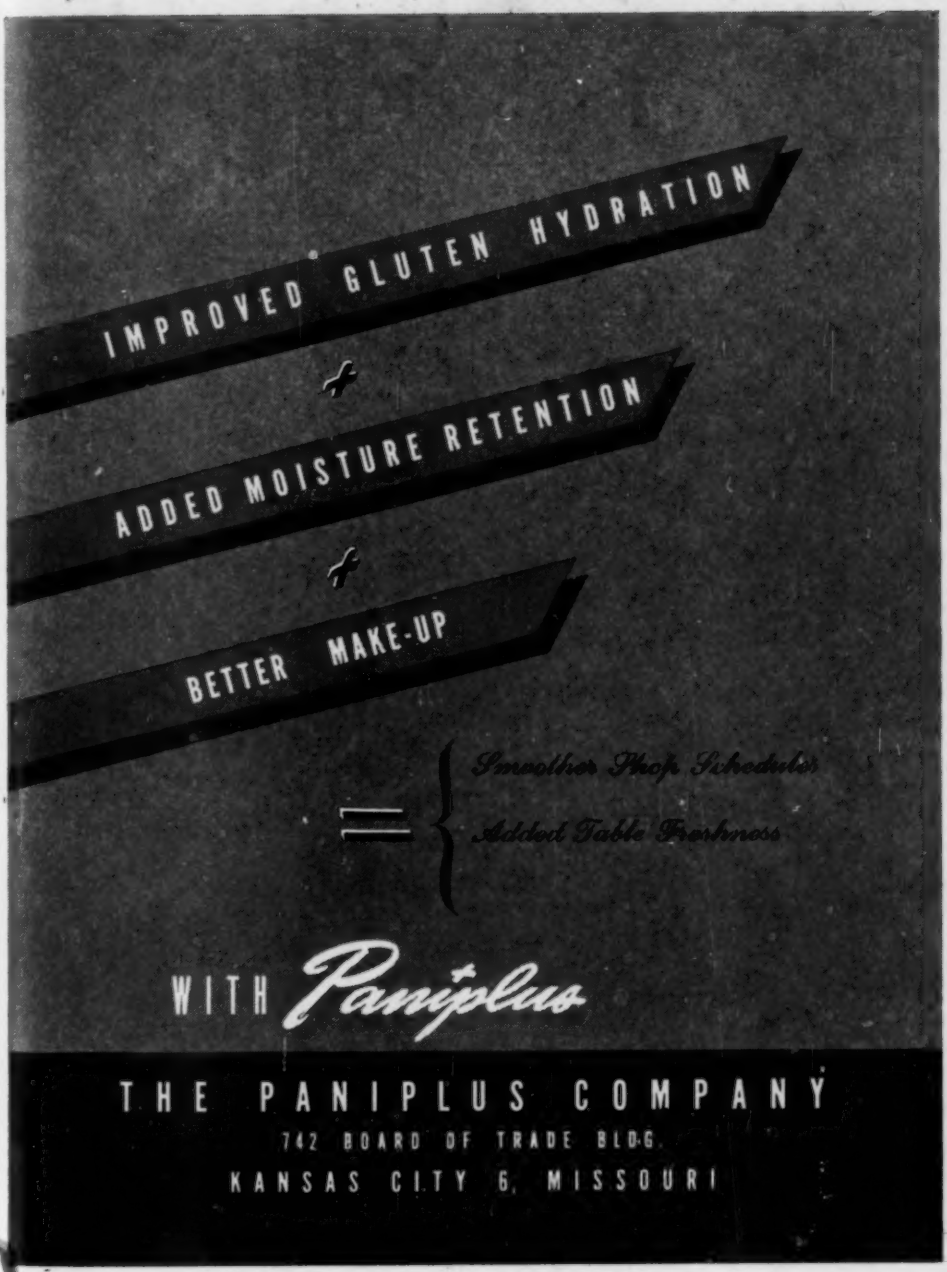
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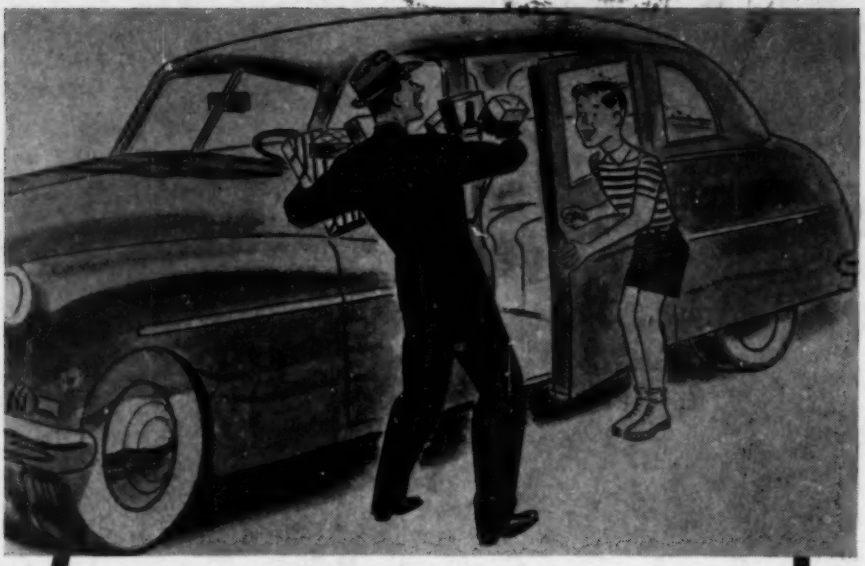

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